UNIVERSITY OF MICHIGAN
JUL 15 1952
SCIENCE LIBRARY

VOLUME 42 JUNE, 1952 NUMBER 6

BOT

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

CONTENTS

Carnation mosaic virus: properties and electron microscopy
RALPH W. AMES AND H. H. THORNBERRY 289
Bacterial disease of wheat caused by Corynebacterium tritici (Hutchinson) Bergey et al
R. S. VASUDEVA AND M. K. HINGORANI 291
The production and role of antibiotics in the soil. III. Terramycin and aureomycin
NORMA MARTIN AND DAVID GOTTLIEB 294
The influence of tuber development on scab infection in Katahdin potatoes
J. K. RICHARDSON 297
A halo-producing strain of Pseudomonas glycinea DONALD W. CHAMBERLAIN 299
Preparation of slide-mounts to study distribution of preservative material in wood (A phytopathological note) DAVID H. MARSDEN 300
Studies on resistance to cucumber virus 1 in spinach
GLENN S. POUND AND PEN-CHING CHEO 301
Relation of air temperature, soil temperature, photoperiod, and light intensity to the concentration of cucumber virus 1 in spinach
PEN-CHING CHEO AND GLENN S. POUND 306
Root injury as a factor in the assessment of chemotherapeutants
W. G. KEYWORTH AND A. E. DIMOND 311
Relation of the black rot pathogen to cabbage seed
A. A. COOK, R. H. LARSON, AND J. C. WALKER 316
Bacterial chalk rot of yellow and pink callas
PETER A. ARK, MORTIMER P. STARR, AND DONALD D. SUTTON 320
Distribution of potato late blight around inoculum sources PAUL E. WAGGONER 323
Cowpea as an assay host for cucumber virus 1
W. H. SILL, JR. AND J. C. WALKER 328
Effect of certain antibiotics on lesion production by two plant viruses
CURT LEBEN AND R. W. FULTON 331
Cantaloupe powdery mildew control with dinitro capryl phenyl crotonate G. H. GODFREY 335
Phytopathological notes:
The antibiotic activity of thiolutin in the chemotherapy of the Fusarium wilt of tomato K. S. GOPALKRISHNAN AND J. A. JUMP 338
Transmission of a leaf mosaic associated with color break in the flowers of Dendrobium superbum Reichb. f. HARRY H. MURAKISHI 339
Report and abstracts of the ninth annual meeting of the Potomac Division of the American Phytopathological Society 340
NEWS

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

DuPont Announces

MANZATE
TRADE MARK
FUNGICIDE

for TOMATOES and POTATOES

Based on Manganese Ethylene Bisdithiocarbamate

EXPERIMENTAL QUANTITIES NOW AVAILABLE

"Manzate", sixth in a series of dithiocarbamate fungicides offered by Du Pont, has special advantages on tomatoes and potatoes. It has already given a highly satisfactory account of itself in Florida this year where it has been evaluated in carefully conducted tests and has also been used by commercial growers. This substantiates results already obtained in several years' tests in 32 states, Hawaii, Canada and Mexico.

FOR TOMATOES—"Manzate" fungicide is a single material effective against all the major fungous diseases of tomatoes. Now a program is offered employing "Manzate" alone to control diseases instead of alternate chemicals in separate sprays. "Manzate" is effective against early and late blight, anthracnose, gray leaf spot (Stemphylium), and Septoria leaf spot.

FOR POTATOES—"Manzate" is particularly effective against both early blight and late blight, especially when both these diseases are severe at the same time.

At present, Du Pont's recommendations for use of "Manzate" apply only to tomatoes and potatoes. Experimental quantities of "Manzate" fungicide are now available for research purposes. "Manzate" will be tested in 1952 on many other vegetable and fruit crops. For further information, write Du Pont, Grasselli Chemicals Department, Wilmington, Delaware.

DU PONT
REG. U.S. PAT. OFF.

BETTER THINGS FOR BETTER LIVING
. . . *THROUGH CHEMISTRY*

CARNATION MOSAIC VIRUS: PROPERTIES AND ELECTRON MICROSCOPY [1]

Ralph W. Ames [2] and H. H. Thornberry [3]

Carnation mosaic is the most widespread of the carnation virus diseases and the most insidious in its attack. Symptoms are not evident at the time cuttings are normally taken, and the effects of the disease are so obscure that they may pass unnoticed until the stock is completely infected and unfit for propagation. To the direct loss by gradual decline in quantity and quality of flowers the time and money spent in trying to offset these effects may be added. It was hoped that studies on the properties and characteristics of the virus might have practical application in the control of the disease. Before purification of the virus was attempted, it was necessary to know the optimal pH for infection on the assay plants and the range of pH in which the virus would remain active sufficiently long to permit purification. These characteristics, by necessity, were determined with a minimal number of assay plants. One-tenth molar phosphate buffer was selected for the assay of virus without determining the optimal concentration. This paper reports the results obtained.

METHODS AND MATERIALS.—The virus was assayed by inoculations of healthy plants of *Dianthus barbatus* L. using carborundum as an aid in infection. The plants were washed with water immediately after inoculation.

Infectious juice for studies other than purification was prepared as follows: Fresh diseased tissues of the variety King Cardinal were ground through a food grinder. To these macerated tissues, 0.1 molar phosphate buffer at pH 7.2 was added (5 ml. for each 20 gm. of fresh tissue) and the material then strained through a single layer of cheesecloth. This liquid is hereafter referred to as the infectious juice. Fresh healthy tissues were treated similarly for controls.

Material for ultracentrifugation, requiring a preliminary clarification, was processed as follows: The infectious juice (200 ml.) was adjusted to pH 4.0 with 1 normal hydrochloric acid, with vigorous stirring, and filtered through a Celite (No. 209) filter cake with suction. The filtrate at pH 4.0 was discarded. The Celite filter cake was previously prepared on a filter paper base in a 75-mm. Buchner funnel by filtering a slurry of 10 gm. of Celite suspended in water. Such a cake retains less than 10 ml. of liquid in the pores. The material lodged and adsorbed on the Celite cake was dissolved and eluted by filtering 100 ml. of 0.1 molar phosphate buffer at pH 7.2 through the filter cake. The filtrate was returned to the Celite cake and refiltered twice in order to bring about thorough solution and elution of the virus from the Celite. The final filtrate was then centrifuged. A sample of healthy carnation tissues was treated in a similar manner.

The sedimentation procedure consisted of 3 centrifugations at approximately 0° C. The first was at approximately 50,000 × gravity (60 ml. of the Celite filtrate at pH 7.2) for 30 min. to sediment the virus from the slightly viscous liquid. The supernatant was discarded. The sediment (less than 0.5 ml.) was resuspended in 12 ml. of 0.1 molar phosphate buffer at pH 7.2 and allowed to stand overnight at about 3° C. The liquid containing the suspended virus was carefully decanted and centrifuged at 580 × gravity for 15 min. to sediment the coarse particles from the liquid. The supernatant was then centrifuged at 15,000 × gravity for 15 min. to sediment the virus. Sediments (less than 0.1 ml.) from this centrifugation were examined in the electron microscope. Extracts from diseased and healthy tissues were centrifuged simultaneously in separate tubes.

The electron microscope procedure was as follows: Sediments from the final ultracentrifugation of extracts of diseased and healthy carnations were suspended separately in 2 drops of 0.1 molar phosphate buffer at pH 7.2. Approximately 0.001 ml. of the resuspended sediment was then mounted on a collodion film supported by a conventional 200-mesh grid, dried slowly using mild heat, dipped in carbon tetrachloride for 30 to 60 sec. (to harden the collodion film), immersed in a water bath for 10 min., dried on a hot plate at approximately 75° C., and shadowed with palladium at an angle of 4 to 1 (25° angle). The specimen was then ready for examination.

EXPERIMENTS AND RESULTS.—The thermal inactivation time of carnation mosaic virus, determined by heating sealed tubes of juice from diseased carnations in a constant-temperature water bath, was 15 min. at 63° to 68° C. Inactivation of the virus in cuttings by means of hot water has been unsuccessful because of injurious effects on the plant. The results are essentially in agreement with those of Jones[4] and Thomas.[5] Thomas[5] also reported inactivation of carnation mosaic virus *in vivo* within 14 days at 40° C. average.

For guidance in subsequent assay of virus, it was desirable to know the most suitable hydrogen-ion con-

[1] Accepted for publication December 20, 1951.

Part of a thesis presented to the Graduate School of the University of Illinois for the requirements for degree of Doctor of Philosophy in Plant Pathology. Acknowledgment is gladly given to A. E. Vatter for the technical aspects of the electron microscopy, to John J. Scholz for the technical aspects of the ultracentrifugation, and to F. F. Weinard for the technical aspects of carnation culture and the services provided.

[2] Associate Pathologist, Illinois State Natural History Survey, Urbana, Illinois, formerly Graduate Fellow, University of Illinois, Urbana, Illinois.

[3] Associate Professor of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Illinois.

[4] Jones, Leon K. Mosaic, streak, and yellows of carnation. Phytopath. 35: 37-46, 1945.

[5] Thomas, W. D., written communication, February 22, 1950.

centration for infection. To this end, virus samples adjusted to various pH values from pH 3 to pH 10 were inoculated to assay plants. The results indicated that infection may occur when plants are inoculated with virus samples adjusted to pH values between 4.0 and 9.0, but that the most desirable pH range for infection is from 5.0 to 8.0. Virus assay throughout the remaining studies was carried out at pH 7.0.

Effects of hydrogen-ion concentration on infectivity of carnation mosaic virus were determined in the range pH 3.0 to pH 10.0 in 0.1 molar phosphate buffer. Extracts of mosaic infected plants were adjusted to the pH values and held for 6 hr. at room temperature. All samples were then adjusted to pH 7.0 for inoculation. The virus was not completely inactivated when held at pH 4.0 to 9.0 for 6 hr., but was inactivated when kept at pH 3.0 or at pH 10.0 for the same time.

Concentration of carnation mosaic virus in the plant was determined by inoculating indicator plants with infectious juice diluted with 0.1 molar phosphate buffer at intervals up to 1:100,000. Infectious juice from young shoots and from flowers was used in the dilutions. The highest dilution of leaf juice remaining infectious was 1:1000; that of flower juice was 1:500.

Electron microscopy of the amorphous material obtained by a chemical method of purification (acid-ammonium sulfate precipitation) revealed no evidence of virus particles.

Preliminary observations and electronmicrographs of the sedimented material were made on an RCA EMC-2 electron microscope at 30 kilovolts at a working magnification of 5,000 diameters. Electronmicrographs were also taken at 10,000 to 15,000 diameters and the fields were observed at magnifications approaching 36,000 diameters on the RCA EMB electron microscope at 50 kilovolts.

Particles present in partially purified material from diseased carnations (Fig. 1) were numerous and well distributed over the area of the microscopic field and appeared spherical with a diameter of approximately 31 mμ[6]. Particle size was determined by measuring 72 particles at a projected magnification of 400,000 and then calculating the arithmetic mean. No particles were found in the material from healthy carnations.

Plants of *Dianthus barbatus* L. inoculated with the sedimented preparations containing the 31 mμ particles developed typical symptoms of carnation mosaic. Plants inoculated with the material in which the particles were not evident failed to develop symptoms of the disease. The inoculum consisted of the 15,000 $\times$ gravity sediments suspended in 10 ml. of 0.1 molar phosphate buffer at pH 7.2. Any original infectious plant juice that could have been carried into the inoculum would have been diluted at least 1:24,000. Since this dilution greatly exceeds the 1:1,000 dilution capacity of the original infectious juice, it is evident that the infectious entity was purified and concentrated significantly by the procedure.

[6] Ames, Ralph W., A. E. Vatter, John J. Scholz, and H. H. Thornberry. Partial purification of carnation-mosaic virus. (Abstr.) Phytopath. 41: 1, 1951.

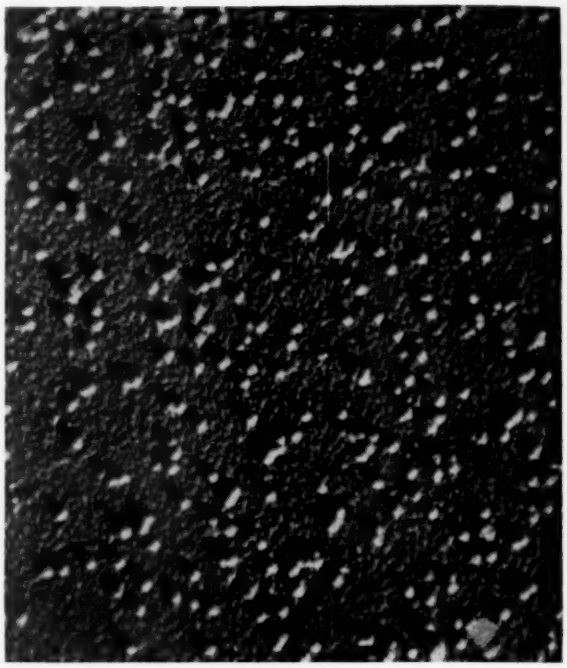


FIG. 1. Electronmicrograph of palladium-shadowed carnation mosaic virus, concentrated and partially purified by adsorption on Celite at pH 4.0 and elution at pH 7.2, preliminary sedimentation at 50,000 $\times$ gravity, clarification sedimentation at 580 $\times$ gravity, and final sedimentation of particles at 15,000 $\times$ gravity. Magnification of approximately 9,000 diameters enlarged to approximately 56,000.

The spherical particles approximately 31 mμ in diameter appear to be the infectious entity of carnation mosaic virus since the dilution of any original infectious liquid accompanying the sediments would have greatly exceeded the infectivity of the original juice. In addition, the sediments from the juice of diseased plants contained the particles and were infectious, whereas sediments from juice of healthy tissues did not contain the particles and were not infectious.

SUMMARY

The thermal inactivation time of carnation mosaic virus *in vitro* is 15 min. at 63° to 68° C.

The most effective H-ion concentration for infection of the test plants (*Dianthus barbatus* L.) is about pH 7.0.

The virus in expressed juice was stable for 6 hr. at room temperature over the range pH 4.0 to pH 9.0.

The dilution end-point of virus in infectious leaf juice was 1:1,000, and in infectious flower juice it was 1:500.

The virus could be sufficiently purified and concentrated by adsorption and elution, along with dif-

ferential centrifugation, to be detected in the final sediments at 15,000 × gravity. These final sediments revealed spherical virus-like particles by electron microscopy. The particles, thought to be the virus, have a diameter of approximately 31 mμ. Samples containing these particles were infectious.

ADDENDUM

Since the submission of this manuscript and its acceptance for publication, a paper[7] by Noordam, Thung, and van der Want on a carnation mosaic virus has come to our attention. They report that the virus has a sedimentation constant of 108×10^{-13} or a molecular weight of about 6,000,000, that the particles are spherical and about 33 mμ in diameter, that the dilution end-point of the original infectious juice is beyond 10^{-5}, and that the thermal inactivation point is between 80 and 90° C. Although these particles appear to be similar in size and shape to the particles we obtained, the 2 viruses differ in thermal inactivation and dilution end-point of the original infectious juice. The 2 viruses could be either related strains of 1 virus or 2 distinct viruses.

[7] Noordam, D., T. H. Thung en J. P. H. van der Want. Onderzoekingen over anjermozaiek, I. Investigations on carnation mosaic, 1. Tijdschr. Pl.-ziekten 57: 1-15. 1951.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

BACTERIAL DISEASE OF WHEAT CAUSED BY CORYNEBACTERIUM TRITICI (HUTCHINSON) BERGEY ET AL.[1]

R. S. Vasudeva and M. K. Hingorani

The bacterial disease of wheat locally known as tundu and caused by *Corynebacterium tritici* (Hutchinson) Bergey et al. was first reported from India by Hutchinson(7) and later from Egypt by Fahmy and Mikhail(6), from western Australia by Carne(2), and from China by Cheo(4).

The disease is characterized by narrow white flecks on the upper leaf blade and by a bright yellow slime or gum, which is a mass of bacteria, formed on abortive ears and the leaves in contact with them (Fig. 1) while the ears are still in the boot. The gum forms sticky layers between the glumes and between the stem and the sheath, interfering with the growth of the plant so that the stem becomes distorted. When heads have been out for a few days and the atmosphere is dry, the slime becomes hard and brittle; otherwise it can be seen trickling down. Often diseased spikes become curled and distorted before heading.

The loss due to this disease has not yet been assessed, but as the affected ears fail to yield any grain, its appearance even in a mild form is responsible for considerable loss to an individual cultivator. In Delhi State the damage can be safely put at about 1 to 2 per cent on an average, but in individual cases it may be as high as 50 per cent. The disease is widespread in Delhi State and in certain areas of the Punjab.

EXPERIMENTAL WORK.—Pure cultures of a bacterium were obtained invariably by dilution-plate isolations from diseased specimens. Morphologically and physiologically the bacterium corresponded with the description of *Corynebacterium tritici* (1). Although the characters of this organism would place it in Pseudomonadaceae as *Pseudomonas tritici* Hutchinson, according to the latest edition of Bergey's Manual (1) Burkholder and Starr think that it is most closely related to other more typical Corynebacteria and therefore it has been named *Corynebacterium tritici* (Hutchinson) Bergey et al.

Inoculation tests.—Young potted seedlings of a susceptible local variety of wheat extensively grown in Delhi State and field-grown plants under humidity tents were inoculated with pure cultures of the bacterium. In another experiment, diseased ears were incorporated into several pots of soil before seed was sown. Bell jars were inverted over the pots upon seedling emergence to maintain high humidity. These experiments were repeated every month from October to March but no infection was observed.

To determine whether wheat plants could be predisposed by exposure to high relative humidity before inoculation with the bacterium, three pots of wheat were placed in a humidity tent, and three control pots kept on the greenhouse bench, for 48 hr. Both sets of plants were then inoculated, by spraying and by hypodermic injection, and placed in the humidity tent for 48 hr. after which they were kept out of doors. All these attempts to reproduce the disease were unsuccessful.

Milne(9) studied earcockle in wheat caused by a nematode, *Anguillulina tritici* (Steinbuch) Gervais & v. Beneden, and found bacterial growth along with gall formation in the ears. Fahmy and Mikhail(6), Carne(2), and Cheo(4) also showed that the bacteria were carried into the plants by nematodes. Chaudhury(3), however, reported that bacteria alone could cause the disease and the presence of nematodes was not necessary.

Properly controlled field and pot experiments were.

[1] Accepted for publication January 20, 1952.

FIG. 1. Center, four diseased heads resulting from the addition of nematode galls to the soil. (Left, two control heads; right, two heads from plants inoculated with the bacterium.)

therefore, made to determine the role of nematodes in the development of the disease. Seeds of susceptible wheat were mixed with nematode galls in equal numbers and sown. Typical symptoms were obtained (Fig. 2), and the average percentage of infection ranged from 40 to 70.

Effect of environment.—Hutchinson (7) mentions that the bacterial disease is one of badly drained areas and that high soil temperature reduces the number of bacteria surviving in the soil to a minimum, owing to the comparatively low thermal death point of *Corynebacterium tritici*. Wheat seeds were mixed with nematode galls in equal numbers and sown in eight plots, each having ten 5-ft. rows. Four rows were given normal irrigation every 20–25 days, the other four receiving one extra flooding 2 days after each normal irrigation. Two plots from each series were sown on October 25 and two on November 25. Mean minimum and maximum air temperatures for the 2 years of the experiments are in table 1 and data regarding incidence of the disease in plots which received various amounts of irrigation during October and November sowings are in table 2.

TABLE 1.—*Mean minimum and maximum temperatures during the experiment*

	Mean minimum temperature (°F.)		Mean maximum temperature (°F.)	
Month	1949–1950	1950–1951	1949–1950	1950–1951
October	62.2	60.1	90.2	89.7
November	46.2	47.2	80.6	80.2
December	41.4	39.2	71.8	69.9
January	46.7	43.3	70.8	67.3
February	42.6	43.4	69.7	73.2
March	53.9	54.7	81.2	82.2

FIG. 2. Results of inoculation tests with nematode galls and the bacterium.

TABLE 2.—*Effect of moisture and time of sowing on disease incidence*

	Percentage infection in plots			
	Normally irrigated		Flooded	
	I	II	I	II
1949-1950				
October	30	28	65	60
November	11	7	18	12
1950-1951				
October	21	22	55	45
November	12	15	18	19

The data indicate that the disease was more severe in October sowings and was further aggravated by flooding. Incidence of the disease in general was lower in November sowings and flooding did not appear to have so marked an effect on the severity of the disease. In cultivators' fields the disease was more severe during January and February, when the temperature was low, than in March and April. It was also more prominent in patches around wells and water channels where soil moisture was high.

Control.—Since infection takes place mainly through the nematode galls that are mixed with the seed, a control program for this disease must be based on their elimination. Seed treatment that will serve this purpose and at the same time is simple and cheap to apply appears to afford the most practical method of control. Chu(5) has reviewed the literature on this aspect in detail.

The galls can be only partially removed by screening and fanning so that this method is not satisfactory. Sedimentation by salt brine (40 lb. salt to 25 gal. water), flotation in water, and Luthra's solar treatment (8) were, therefore, tried and the results are in table 3. Small plots were used, each having fifteen 8-ft. rows. There were four replications for each treatment and nontreated seeds with galls were sown for controls. Maximum ground temperature during the solar treatment was 133.7° F. and maximum air temperatures at 1 in. and 6 in. above ground were 116.6° and 111.2° F., respectively.

TABLE 3.—*Effect of different seed treatments on disease incidence*

Treatment	Percentage infection in plots:			
	I	II	II	IV
Control	31	34	24	23
Solar heat	21	32	20	17
Flotation in water	0	0	0	2
Sedimentation by salt brine	0	0	0	0

The data indicate that where the galls were removed by sedimentation or by flotation the disease was kept under control, whereas solar treatment was not effective in destroying the bacteria in the nematode galls.

SUMMARY

Corynebacterium tritici (Hutchinson) Bergey et al. is unable to cause the disease in the absence of the nematode, *Anguillulina tritici* (Steinbuch) Gervais & v. Beneden.

Time of sowing and soil moisture appreciably affect incidence of the disease.

Control can be successfully effected by removal of galls by sedimentation or flotation in water.

INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI, INDIA

LITERATURE CITED

1 BREED, ROBERT S., E. G. D. MURRAY, AND A. PARKER HITCHENS. Bergey's manual of determinative bacteriology. Sixth ed. 1529 pp. Williams & Wilkins Co., Baltimore, Maryland. 1948.

2. CARNE, W. M. Earcockle (Tylenchus tritici) and a bacterial disease (Pseudomonas tritici) of wheat. Jour. Dept. Agr. W. Aust. **3**(2): 508-512. 1926.

3. CHAUDHURY, H. A bacterial disease of wheat in the Punjab. Proc. Ind. Acad. Sci. **1**: 579-585. 1935.

4. CHEO, C. C. A note on the relation of nematodes (Tylenchus tritici) to the development of the bacterial disease of wheat caused by Bacterium tritici Hutchinson. Ann. Appl. Biol. **33**: 446-449. 1946.

5. CHU, VONG-MAY. The prevalence of the wheat nematode in China and its control. Phytopath. **32**: 288-295. 1945.

6. FAHMY, T., AND T. MIKHAIL. The bacterial disease of wheat caused by Pseudomonas tritici Hutchinson. Agr. Jour. Egypt (New Ann. Ser. 1923) **1**: 64-72. 1925.

7. HUTCHINSON, C. M. A bacterial disease of wheat in the Punjab. Mem. Dept. Agr. India Bact. **1**: 169-175. 1917.

8. LUTHRA, J. C. Solar treatment of wheat loose smut. Indian Fmg. **2**: 416-418. 1941.

9. MILNE, D. Earcockle in wheat. Bul. Dept. Agr. Punjab Bot. **1**: 1-5. 1919.

THE PRODUCTION AND ROLE OF ANTIBIOTICS IN THE SOIL
III. TERRAMYCIN AND AUREOMYCIN [1]

Norma Martin and David Gottlieb [2]

Studies reported in this paper on terramycin and aureomycin are part of an extensive investigation on the production and role of antibiotics in soil. They were designed primarily to determine whether antagonisms of micro-organisms toward each other in soil could be ascribed to the production of antibiotics. Such data are patently important in the use of antibiotic-producing organisms for disease control. An historical survey of the literature has appeared in earlier publications of this series (6, 7). Two previous papers have dealt at length with streptomycin and Chloromycetin (6, 7) and other shorter reports on the activity of actidione, clavacin, and streptothricin in the presence of clay or soil have been published (2, 3, 5, 8). The first of these studies showed that streptomycin is adsorbed on clays and is not biologically active in sandy or in silt loam soils or even in soils of low clay content such as peat. Because of the marked inactivation of this antibiotic by soil its production in this substrate could not be demonstrated (9). More recent studies with streptomycin dependent strains, which can detect this antibiotic even when it is not active in soil, failed to reveal production of the material by *Streptomyces griseus* in soil.[3] The second study showed that chloromycetin, a neutral compound, remained active in sterile soil. Small amounts of the antibiotic were produced by the actinomycete growing in otherwise sterile and unamended soil, but these small amounts did not inhibit the growth of *Bacillus subtilis*. Furthermore, the antibiotic was readily decomposed by the flora in normal soil (7). Studies with clays showed that the other basic antibiotics are readily adsorbed while neutral and acidic ones are only slightly inactivated (3, 8).

Unlike antibiotics previously investigated, terramycin and aureomycin are amphoteric. Therefore, their activities in soil might be dependent on the hydrogen ion concentration of the milieu. Four approaches have been followed in studying the antibiotic-soil complex; (a) effect of clays on activities of the antibiotics, (b) effect of soil on activities of the antibiotics, (c) population changes effected by the actinomycetes producing such materials, and (d) production of the antibiotics in soil.

MATERIALS AND METHODS.—To study the effects of clay and soil on the antibiotics two clays were used, a bentonite and an illite clay, and a black prairie-loam soil. These materials and the general methods have been described previously (9). Terramycin and aureomycin were the commercially available hydrochloride salts. *Streptomyces rimosus* and *S. aureofaciens*, which produce these antibiotics respectively, were used for studies on the production of the compounds in soil. All assays were made by methods similar to those of Loo et al. (4). *Brucella abortus* was the test organism for terramycin (1) and *Bacillus subtilis* on nutrient agar at pH 6.0 was the test organism for aureomycin. Both assays were sensitive to 5 μg. per ml. To study antibacterial activities of the antibiotics and their producers in soil, indigenous soil bacteria were chosen, *Bacillus polymyxa* for terramycin and *B. subtilis* for aureomycin; these organisms are sensitive to 1 μg. of the antibiotic per ml. of broth.

Most of the techniques were similar to those previously reported for streptomycin and Chloromycetin (6, 7). Because of its amphoteric nature, however, the studies with terramycin were carried out at a number of pH levels using Clark and Lubs buffers as solvents. The buffers served to modify the compounds when added to soil; in the acid range the antibiotic was predominantly basic, but in the basic range it acted predominantly as an acid. The exact pH of change depends on the isoelectric point of each material. Varying the H-ion concentration permitted determination of whether inactivation occurred more readily when the antibiotic was in its basic or acidic form. In biological studies, buffer solutions alone were always used as controls.

RESULTS.—*Effect of clays on the activities of the antibiotics.*—Clays readily adsorb both terramycin and aureomycin. Flocculation of illite clay begins at a concentration of 12,000 μg. of terramycin per gram of clay, when this antibiotic is acting as a base (in buffer at pH 2.2). When terramycin is in the acid form (in buffer at pH 8.2) however, no flocculation occurs even with concentrations as high as 500,000 μg. On the other hand, adsorption, as measured by bioassay, occurs with both ionic types. With the acid type a saturation level is reached at 30,000 μg. per gram of clay but with the basic type the adsorption continues up to 40,000 μg. at least. A straight line relationship exists between concentration of terramycin and its adsorption by illite clays up to the saturation point. Complete adsorption does not occur even below the saturation point because of an equilibrium between the amount adsorbed and that remaining in solution. For both ionic forms of terramycin, the equilibrium constant, or K value, is approximately 0.8 with illite clay. Bentonite has a much greater adsorptive capacity, for the saturation level is not reached even at 60,000 μg. per gram of clay. The K values for the acidic and basic forms with bentonite are 0.77 and 0.95 respectively. Because of the instability of the acid ion of aureomycin, only the basic form of this

[1] Accepted for publication February 8, 1952.

[2] Respectively, Research Assistant and Associate Professor of Plant Pathology, Department of Horticulture, University of Illinois, Urbana, Illinois.

[3] Personal communication from Dr. P. Siminoff.

antibiotic was studied. With the illite clay, flocculation occurs above 12,000 μg. per gram of clay. Adsorption takes place far below this level and continues to 40,000 μg. per gram of clay. As with terramycin, bentonite is not flocculated but adsorbs aureomycin linearly up to a concentration of 100,000 μg. per gram of clay. With aureomycin, too, an equilibrium exists between the amount adsorbed and that remaining in solution. The K value is 0.6 for illite and 0.94 for bentonite.

Effect of soil on the activities of the antibiotics.—The inactivating effect of soil on the inhibitory properties of terramycin was readily demonstrated. Black prairie loam soil removed the antibiotic from solution at four levels from pH 3.2 to pH 7.6. Greater amounts were removed by soil when the terramycin solution was at a pH below the isoelectric point of the antibiotic than at higher levels. Approximately 4,000 μg. of terramycin at pH 3.2 and pH 4.9 could be added to a gram of air-dried soil before any free antibiotic could be detected in solution. At pH 5.6 and pH 7.6, terramycin could be detected when 400 μg. per gram of air-dried soil was added, but not when a lesser amount was added. In the latter case, nevertheless, free terramycin at a concentration below the sensitivity of the assay might have been present.

Even though terramycin was removed from the soil solution it could possibly be active in an adsorbed state. Terramycin was added at concentrations of 100, 200, 500 and 1000 μg. per gram of air-dried soil. *B. polymyxa* was added to the soil at 15,000 cells per gram and the bacterial populations were counted after 5 days incubation at 30° C. in soil at pH 5.6, and after 16 days in soil at pH 6.2. The data (Table 1) show that *B. polymyxa* was inhibited by 500 μg. of terramycin per gram of soil at pH 5.6 and by 200 μg. of terramycin per gram of soil at pH 6.2. It should be noted, however, (a) that pH of the soil modifies the concentration necessary for inhibition of the sensitive organism and (b) that at neither pH of the soil solution was terramycin effective at as low a concentration as 100 μg. per gram of soil, an amount higher than that expected to occur in soil.

Absence of inhibition by aureomycin in soil is still more striking. *B. subtilis*, which is sensitive to 1 μg. per ml. of broth, was not inhibited by concentrations as high as 720 μg. per gram of soil at pH 5.6.

TABLE 1.—*Inhibition of B. polymyxa by terramycin in soil*

μg. of terramycin	Population of B. polymyxa x 10^{-6} per gram of soil	
	pH 5.6	pH 6.2
0	–	380
100	–	360
0	18	330**
200	19	23**
0	15 **	360**
500	7.6**	50**
0	17 **	–
1000	3 **	–

**Difference between means of 6 counts significant at 0.01 per cent level.

Population changes effected by S. rimosus and S. aureofaciens.—Three types of experiments were made to determine whether sufficiently high concentrations of antibiotics would be produced by *S. rimosus* growing in sterile soil to show antagonistic effects: 1) *S. rimosus* was added 4 wk. prior to addition of *B. polymyxa*, 2) both organisms were added simultaneously and 3) *B. polymyxa* was added 7 days prior to addition of *S. rimosus*. In all 3 experiments the mean differences between *B. polymyxa* populations in the control and test material were highly significant at the 1 per cent level (Table 2).

TABLE 2.—*Effect of S. rimosus on growth of B. polymyxa and of S. aureofaciens on B. subtilis in soil*

Experiment	Populations x 10^{-6}* (means of duplicate counts on 3 samples)			
	Antagonist		Sensitive Organism	
	Alone	In Combination	Alone	In Combination
1) *S. rimosus* added 30 days prior to *B. polymyxa*	46	58	18	6.2
2) Both added at the same time	47	43	1210	665
3) *B. polymyxa* added 7 days prior to *S. rimosus*	–	816	1210	393
4) *S. aureofaciens* added 14 days before *B. subtilis*	118	101	7.0	1.1

*The counts in series 1 were made 7 days after inoculation with *S. rimosus*. In series 2 and 3 the counts were made 23 and 25 days after the addition of *S. rimosus*.

The antagonistic effect of *S. aureofaciens* on *B. subtilis* in soil was similarly investigated. Only 1 series was run, that in which *S. aureofaciens* was established in the soil 14 days before addition of *B. subtilis* (Table 2). The difference again was highly significant, showing that the antagonist inhibited the sensitive organism.

Production of the antibiotic in soil.—No satisfactory method was found for detection of terramycin and aureomycin in soil in the bound state. Even at levels of 200 μg. of antibiotic per gm. of soil no activity was recovered. Extractions were tried using water, 0.05 N HCl, 0.1 N HCl, 0.05 N NaOH, 0.1 N NaOH, butanol, and a pyridine-acetic acid salt. It is impossible to say whether or not the antibiotics are actually produced in soil since there was no satisfactory technique for their detection in low concentrations.

DISCUSSION.—From the data obtained in these experiments it is apparent that the presence of the acti-

nomycetes *S. rimosus* or *S. aureofaciens* in soil hinders the growth of the bacteria *B. polymyxa* and *B. subtilis* respectively. Even though both species produce antibiotic in liquid culture media the antagonistic effects in soil cannot be attributed to the production of antibiotics in liquid culture media the antagonistic effects ramycin in soils containing a large population of *S. rimosus* nor could we find aureomycin in soil containing *S. aureofaciens*. Furthermore, when these antibiotics were added to soil in concentrations as high as 100 μg per gm of soil they did not inhibit the growth of susceptible bacteria; in broth these bacteria were inhibited by 1 μg per ml. Both antibiotics are amphoteric substances which tend to act as bases in an acid medium, and are therefore adsorbed by negatively charged clays or might combine with organic acids in soil. Thus, even if they were produced they would not remain free since many soils tend to be acidic. The antagonism observed when the bacterium and actinomycete are grown together in soil can be due to other competitive mechanisms as has been shown for *S. griseus* (6). This organism inhibits *B. subtilis* in soil and also produces streptomycin in laboratory media. Even if the antibiotic synthesizing mechanism of *S. griseus* is destroyed with X-rays, the competitive effects still occur in soil and the growth of *B. subtilis* is prevented in the presence of the actinomycete. In normal soils, which contain a large and diverse number of microorganisms, still another factor must be considered; namely, that these antibiotics might be rapidly broken down by the metabolic activities of the soil microflora. Such degradation occurs when either streptomycin (7) or chloromycetin (5) are added to non-sterile soils.

To be effective in soil an antibiotic must be produced in concentrations great enough to inhibit soil inhabiting microorganisms, should not be adsorbed by soil colloids or combine with other organic materials in soil, and should be refractory to the dissimilatory activity of other soil microflora. Terramycin and aureomycin apparently do not satisfy these conditions. Other investigations in this laboratory have shown that neutral or acidic compounds might better fulfill these requirements since they remain active in sterile soil. If such compounds are produced in soil by the many organisms which have been shown to control diseases of crop plants then the role of antibiotics would be established.

SUMMARY

1. Terramycin and aureomycin are inactivated by illite and bentonite clays.
2. The antibiotics are removed from aqueous solutions by soil.
3. Relatively high concentrations of the antibiotics failed to inhibit sensitive organisms in soil. The amount of antibiotic necessary for the inhibition of a sensitive organism in soil is dependent upon the pH of the soil solution.
4. Antibiotic producing strains of *S. rimosus* and *S. aureofaciens* inhibited sensitive organisms in soil.
5. Extraction procedures failed to recover the antibiotics from soil when these compounds are not free in the soil solution.

LITERATURE CITED

1. Gottlieb, David, Marvin Legator and Betty Bevan. A plate method of antibiotic assay with Mycobacterium tuberculosis 607 and Brucella abortus. Antibiotics and Chemotherapy **1**: 47-48. 1951.
2. ——— and Paul Siminoff. Activity of antibiotics in soil. Proc. 7th. Int. Bot. Congr. (Stockholm 1950) 1950. In press.
3. ———and ———. The role of antibiotics in soil. Phytopath. **40**: 11. 1950.
4. Gottlieb, David and Paul Siminoff. Production and activity of chloromycetin in soil. Bacteriological Proc. 1951. Soc. Amer. Bact. 1951.
5. ——— and ———. The production and role of antibiotics in the soil. II. Chloromycetin. Phytopath. **42**: 91-97 1951.
6. Loo, Y. H., P. S. Skell, H. H. Thornberry, J. Ehrlich, J. M. McGuire, G. M. Savage, and J. C. Sylvester. Assay of streptomycin by the paper-disc plate method. Jour. Bact. **50**: 701-709. 1945.
7. Pramer, D. and R. L. Starkey. Decomposition of streptomycin. Science **113**: 127. 1951.
8. Siminoff, P. and David Gottlieb. The adsorption of antibiotics by natural substrates. Abstr. mimeographed. Illinois Acad. Sci. 1950.
9. ——— and ———. The production and role of antibiotics in the soil. I. The fate of streptomycin. Phytopath. **41**: 420-430. 1951.

THE INFLUENCE OF TUBER DEVELOPMENT ON SCAB INFECTION IN KATAHDIN POTATOES [1]

J. K. Richardson [2]

There is little information on the development of potato scab under field conditions and a wide divergence of opinion regarding the time of infection, the manner of spread in the field, and the possibility of increase in severity with delayed harvesting. The present investigation was initiated for the purpose of clarifying the relation between scab initiation and tuber development under field conditions.

According to Sanford[3] under controlled conditions there seems to be a critical period of susceptibility during the growth of the tuber after which very little scab infection takes place. Fellows[4] showed that under greenhouse conditions tubers enlarge by elongation at the apical end and by increase in diameter. He showed further, that tubers in different stages of growth varied in susceptibility to infection. If tubers were inoculated when plants were nearly mature neither large nor small tubers, but only medium sized ones, became infected. If inoculated when the plants were only partially mature, small tubers remained unaffected while larger ones became infected at the apical end. Infection appeared to be coincident with growth. On the other hand, tubers inoculated under field conditions failed to become infected even when growing rapidly.

Location.—Investigations were carried on during the summers of 1949 and 1950, 2 periods when the precipitation varied sufficiently to classify the seasons as dry and wet respectively. A field of fine, Fox sand was chosen, because of its naturally high scab-producing potentialities, to test the interrelation between different cultural practices and soil amendments and the incidence of potato scab under field conditions.

Procedure.—Potatoes of the Katahdin variety were chosen for the test because of their extreme susceptibility to the disease and their wide distribution throughout the province.

In 1949, sets from scab free tubers were planted in pots of steam sterilized soil in May and transplanted to the field on different dates between July 26 and August 22. When the plants were dug it was observed that those transplanted after August 15 showed only minor tuber development and little infection.

In 1950 the sets were planted early in April to allow sufficient time for substantial tuber development before transplanting and for a period of growth after the plants became established in the field. When plants had produced tubers longer than 1½ in., they were transplanted into the heavily scab-infested soil of the field. On removal from pots, each tuber was marked for future identification and its size recorded.

When plants were harvested just prior to maturity, data on final size of tubers and incidence of scab were recorded and compared with the data originally obtained at time of transplanting. Unmarked tubers, formed after the transplanting, were checked for scab incidence only.

Results and Discussion.—The incidence of scab was calculated as the percentage of tuber surface covered by scab lesions. Of the 60 plants used in 1949, 133 tubers were tagged on the 38 transplanted between July 26 and August 8, and 90 on the 22 transplanted between August 16 and 22. The 1950 test comprised 290 marked tubers on 70 plants, all transplanted into contaminated soil between July 10 and 15.

During harvesting in both seasons, it was noted that many of the smaller, marked tubers had disintegrated and could not be located, while others had grown only slightly, or not at all. In all plants there appeared to be a more decided tendency towards initiation of new tubers than towards enlargement of those marked at the time of transplanting. This was particularly evident in the 1949 late planted series. In spite of these handicaps, sufficient data (Table 1) were obtained to show a correlation between tuber development and scab infection.

Table 1. *Incidence of scab on various groups of tubers at harvest*

	1949		1950	
	Number	Av. Scab	Number	Av. Scab
		per cent		per cent
1. Tubers initiated after transplanting	181	16.9	67	16.4
2. Tubers initiated as secondary growth from marked tubers	—	—	11	18.8
3. Tubers which did not enlarge after being transplanted	68	0.0	19[a]	0.8
4. Tubers which enlarged one inch or less	43	0.3	29	3.0
5. Tubers which enlarged between one and two inches	45	7.2	33	12.9
6. Tubers which enlarged more than two inches	22	15.6	7	10.8

[a] Only one tuber infected.

[1] Accepted for publication February 8, 1952.

Contribution No. 1124 of the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. An investigation carried on as part of the potato scab research project conducted under the supervision of the Ontario Potato Scab Research Committee.

[2] Associate Plant Pathologist, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

[3] Sanford, G. B. The relation of soil moisture to the development of common scab of potato. Phytopath. 13: 231-236. 1923.

[4] Fellows, Hurley. Relation of growth in the potato tuber to the Potato-Scab disease. Jour. Agr. Res. [U.S.] 32: 757-781. 1926.

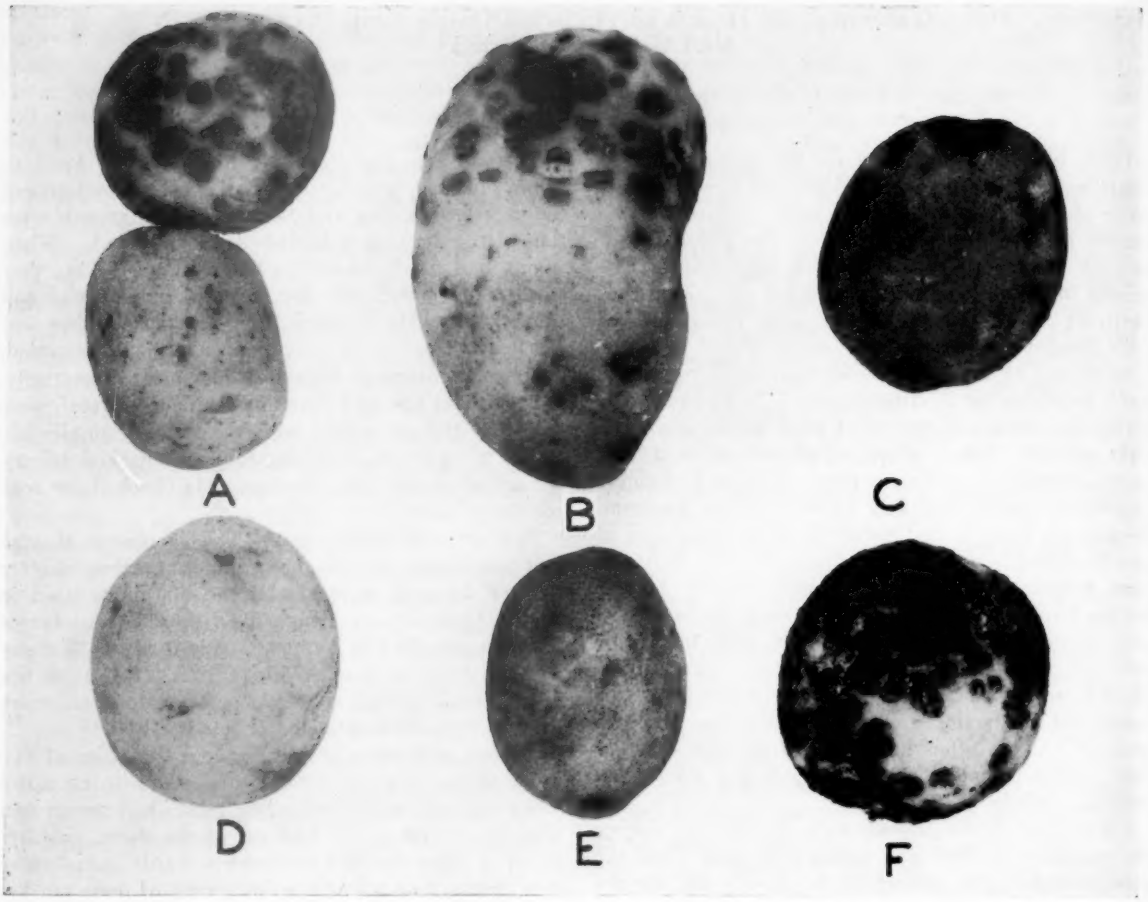


FIGURE 1. A) Transplanted tuber showing russetting but no increase in size. Secondary growth developed after transplanting, severely scabbed; B) Tuber which elongated over two inches showing severe scab only in the areas of growth; C) Tuber which increased less than one inch in length showing infection only at the apical end; D) Healthy non-scabbed tuber; E) Severe russetting only on tuber which did not enlarge after transplanting; F) Severe scabbing on tuber initiated after transplanting.

Tubers initiated after the 1950 transplanting, those from the early transplanted group in 1949, (Fig. 1 F), and all secondary growths of the marked tubers, (Fig. 1 A), were severely scabbed. Wherever tuber enlargement had taken place, the infections were invariably at the apical end of the tuber in the region of new growth (Fig. 1 B).

Tubers which grew only slightly or not at all after transplanting and those parts of tubers which showed no perceptible increase in size became russetted without the development of definite scab pustules (Fig. 1 C & E). Russetting was similar to that occurring on the tubers of many scab resistant varieties growing in the same field and is considered to be the effect of infections on nonsusceptible areas.

In the 1949 late transplanted series, many tubers initiated after transplanting were russetted but had fewer scab pustules than similarly developed tubers of the earlier transplanted series.

Correlation between scab incidence and tuber growth was greater than the data indicate since scab was present only on new growth whereas the percentage scab was calculated in relation to the entire tuber surface.

It is evident that scab infection in the field takes place almost entirely on actively growing tubers or parts of tubers. Thus there is little foundation for the view that the disease will increase in tubers left in the ground after maturity.

SUMMARY

Katahdin potato plants bearing tubers of various sizes were transplanted from sterilized soil to heavily scab infested soil. Tubers produced in contaminated soil and those which increased in size after transplanting developed typical scab lesions. Infection on transplanted tubers occurred only in the area of enlargement and its severity varied directly with tuber growth.

DOMINION LABORATORY OF PLANT PATHOLOGY
ST. CATHARINES, ONTARIO

A HALO-PRODUCING STRAIN OF PSEUDOMONAS GLYCINEA [1]

Donald W. Chamberlain [2]

In June, 1946, the writer's attention was directed to a leafspot on young soybean plants (*Glycine max* (L.) Merrill) in central Illinois. This spot consisted of a small watersoaked or necrotic center surrounded by a pale green to yellow-white halo about 4-7 mm. in diameter. It was commonly termed "halo blight" and believed to be caused by *Pseudomonas phaseolicola* (Burk.) Dowson. A white bacterium was consistently isolated from the lesions.

This organism, designated as BB4, was compared with *Pseudomonas phaseolicola*. Lincoln soybean plants on which the third trifoliate leaf had not yet unfolded were inoculated with BB4; a similar group of plants was inoculated with a culture of *Ps. phaseolicola*. Inoculations were made with an atomizer attached to an air line, spraying the inoculum forcibly against the lower surface of the leaf. Incubation was at 16-18° C. to favor the development of halos.[3]

In 8 days the plants inoculated with *Ps. phaseolicola* showed pale green halos, up to 2.5 cm. in diameter, on the second trifoliate leaves. The first and third trifoliates were not infected. In contrast, the plants inoculated with BB4 had conspicuous yellowish halos, 4-5 mm. in diameter, on the second and third trifoliates, and typical bacterial blight (*Pseudomonas glycinea* (Coerper) Stapp) symptoms on the first trifoliates. A small water-soaked spot was visible at the center of the halo induced by BB4; with *Ps. phaseolicola*, the center of the halo was necrotic with no visible water-soaking.

Cross-inoculations with the 2 organisms were made on Lincoln soybean and on Red Kidney bean, with incubation at 18° C. All plants were in the 2 trifoliate leaf-stage. The reaction of Lincoln to *Pseudomonas phaseolicola* was the same as that of the previous experiment, while Red Kidney bean developed large green-yellow halos. No symptoms appeared on bean plants inoculated with BB4. On soybean, small halos again developed on the second trifoliates and typical bacterial blight symptoms appeared on the first trifoliates. Repeated tests with BB4 on Red Kidney bean produced no symptoms other than occasional necrotic flecking. Soybean plants of various ages, inoculated with the same organism, developed halos only on young leaves and typical blight symptoms on older leaves.

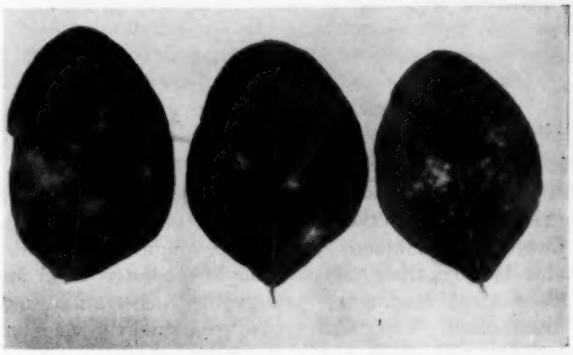


FIG. 1. Effect of temperature on development of halos. Left, leaflet incubated at 10-18.5° C.; center, at 18.5° C.; right, at 18.5-28° C.

Halos were produced on the varieties Illini, Palmetto, Richland, Hawkeye, Earlyana, Gibson, Flambeau, Wisconsin Manchu, Ogden, and CNS.

Lincoln and Illini soybean plants in the 2 trifoliate leaf-stage were inoculated with BB4 and incubated in chambers at the following temperatures: 10° C. night, 18.5° day; 18.5° C. night and day; and 18.5° night, 28° C. day. Relative humidity varied from 40 to 60 per cent. Twelve days after inoculation, the halos measured 5-7 mm. in diameter at the 2 lower temperatures, and 2 mm. at 18.5-28° C. (Fig. 1). Five additional trials confirmed these results. At the higher temperatures the halos lost their identity 2-3 wk. after inoculation and the lesions became indistinguishable from those of typical blight.

In 1947, 1948, and 1949 the halo spot was not found in Illinois. During these years isolates from typical bacterial blight lesions produced no halos in greenhouse tests. In 1949, additional isolates were made from infected leaves from Minnesota, Wisconsin, and Germany, the latter collected by Mr. J. L. Cartter of the U. S. Regional Soybean Laboratory. Only the isolate from Wisconsin produced halos. All isolates, when introduced into the first trifoliate leaves of soybean plants in the 3 trifoliate leaf-stage, produced identical symptoms of bacterial blight. On the second trifoliates, all isolates induced more or less marginal yellowing, most pronounced with the Wisconsin isolate. The German isolate was intermediate, causing more chlorosis than other non-halo-producing isolates.

These experiments indicate that the organism described is a strain of *Pseudomonas glycinea*, differing from the other isolates only in its ability to produce halos on young soybean leaves. The small size of the halos, and the fact that at high temperatures they soon change to lesions typical of common blight might

[1] Accepted for publication January 25, 1952.

[2] Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, in cooperation with the Illinois Agricultural Experiment Station, Urbana, Illinois.

[3] Goss, Robert W. The Relation of Temperature to Common and Halo Blight of Beans. Phytopath. 30: 258-264. 1940.

explain why this type of symptom has not been identified previously with bacterial blight of soybean. Under certain conditions, both halo-forming and non-halo-forming isolates produce appreciable amounts of yellowing around the margins of lesions. Therefore, the symptomatology of bacterial blight cannot be distinguished from that of bacterial pustule on the basis of marginal yellowing, a distinction sometimes used by pathologists.

DIVISION OF FORAGE CROPS AND DISEASES
U. S. DEPARTMENT OF AGRICULTURE
AND
ILLINOIS AGRICULTURAL EXPERIMENT STATION
URBANA, ILLINOIS

A PHYTOPATHOLOGICAL NOTE

Preparation of Slide-Mounts to Study Distribution of Preservative Material in Wood[1]. DAVID H. MARSDEN. Conventional methods of micro-technique are not suitable for preparing thin sections of creosoted wood for histological studies of preservative penetration and distribution. The usual procedure of dehydrating the sections with alcohol, clearing in xylol, and mounting in balsam is not satisfactory because alcohol dissolves certain constituents of the creosote and xylol removes most of the remainder. Substituting the dioxane series for the alcohol and xylol does not help because dioxane also extracts the creosote. Mounting wet sections directly in balsam fails because water and balsam are not miscible. Cutting and mounting dry sections of wood in balsam is difficult and yields mounts quite unsatisfactory for critical study.

The following simple technique solved this problem satisfactorily in histological studies of decayed, creosoted wood. The same method might be used for studies of wood treated with coal tar, creosote-coal tar mixtures, or other colored preservatives and preservative mixtures which are removed by the usual combination of alcohol and xylol.

Thin sections of preservative-treated wood are cut with a sliding microtome in the usual manner. The wood is sectioned without embedding or any kind of softening except for applying water to the surface of the block prior to each cut. Radial and tangential sections of from 5 μ to 30 μ thickness, depending on the species of wood, may be sliced off readily in this manner. Lining up the cut sections temporarily on the knife blade insures accurate identification of serial sections for comparison slides if desired.

One or more sections are immersed in water in a small bottle fitted with a tight rubber stopper and glass tube for attachment to a vacuum pump; the bottle is evacuated for 3 or 4 min., sufficient to remove most air bubbles from the sections; after which the sections may be mounted directly in dark Karo corn syrup. Because corn syrup is entirely miscible with water there is no need for prior dehydration; excess water evaporates from under the cover slip, leaving the section embedded in a thin film of solid, crystal-free corn syrup. When the syrup has hardened, the slide and cover may be cleaned with lightly moistened lens paper or soft cloth. This simple procedure yields permanent mounts; the writer has had some over 2 years still in perfect condition, with the color and location of the preservative essentially unaltered in the wood elements (Figure 1.). The refractive index of Karo is favorable for both microscopy and photomicrography.

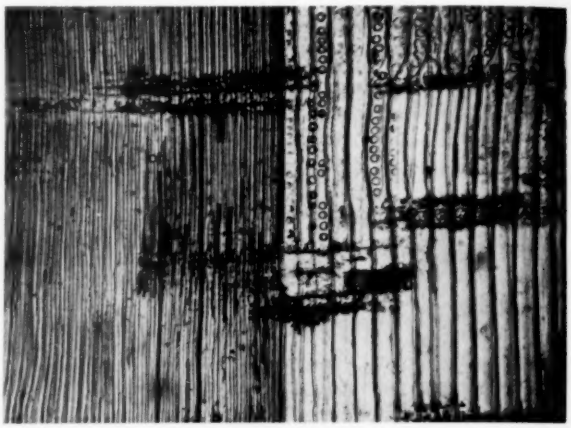


FIG. 1. Photomicrograph of a thin section of creosoted southern pine wood mounted in Karo corn syrup.

For studies of penetration of the wood by fungi and bacteria in relation to distribution of the preservative, it is necessary to make comparison slides of serial sections. One section may be mounted directly in Karo as described above to show the location and density of the preservative, a second serial section may be extracted prior to staining, or may be stained differentially without extracting the preservative and then dehydrated, cleared, and mounted in balsam to reveal the nature and distribution of invading micro-organisms.—Biological Laboratories, Harvard University and Shade Tree Laboratories, University of Massachusetts.

[1] The author is indebted to Dr. W. H. Weston and Dr. M. A. McKenzie for helpful suggestions on the preparation of this note.

STUDIES ON RESISTANCE TO CUCUMBER VIRUS 1 IN SPINACH[1]

Glenn S. Pound and Pen-Ching Cheo

In 1920 Smith[2] reported the development of Virginia Savoy spinach, a variety resistant to "spinach blight" which is incited by cucumber virus 1. The resistance of this variety was derived from a wild Asiatic collection of *Spinacia oleracea* L. By selection from a cross of Virginia Savoy with King of Denmark a second resistant variety, Old Dominion, was developed. Both varieties are savoy in type and have been widely used in the fresh market.

In 1947, a spinach-breeding program was begun at the University of Wisconsin with the purpose of transferring the resistance of Virginia Savoy into smooth-leaved varieties for processing. Early in this study it was learned that when resistant plants were inoculated with cucumber virus 1 and exposed to air temperatures of 28° C. or above, they succumbed rapidly with systemic necrosis. It was important, therefore, to study in detail the effect of temperature and other factors on the expression of resistance to determine the range of environmental conditions most suited for screening and evaluating breeding progenies. Susceptible Nobel spinach was used for comparison in many of the experiments. The identity of the isolate of cucumber virus 1 used was proven by cross protection tests with Price's indicator strain.

[1] Accepted for publication February 29, 1952.
Published with approval of the Director, Wisconsin Agricultural Experiment Station.

[2] Smith, L. B. Breeding mosaic resistant spinach and notes on malnutrition. Va. Truck Exp. Sta. Buls. 31 and 32. 1920.

AIR TEMPERATURE RELATIONS.—In susceptible varieties, spinach blight is characterized by mottling, severe chlorosis, malformation of leaves, and stunting. Disease severity in variety Nobel was found to be directly related to air temperature. The incubation period was shorter and symptoms were more severe at 28° C. than at 24°, 20°, and 16°. Death of infected plants occurred in about 15 days after inoculation at 28° and in about 80 days at 16° (Fig. 1).

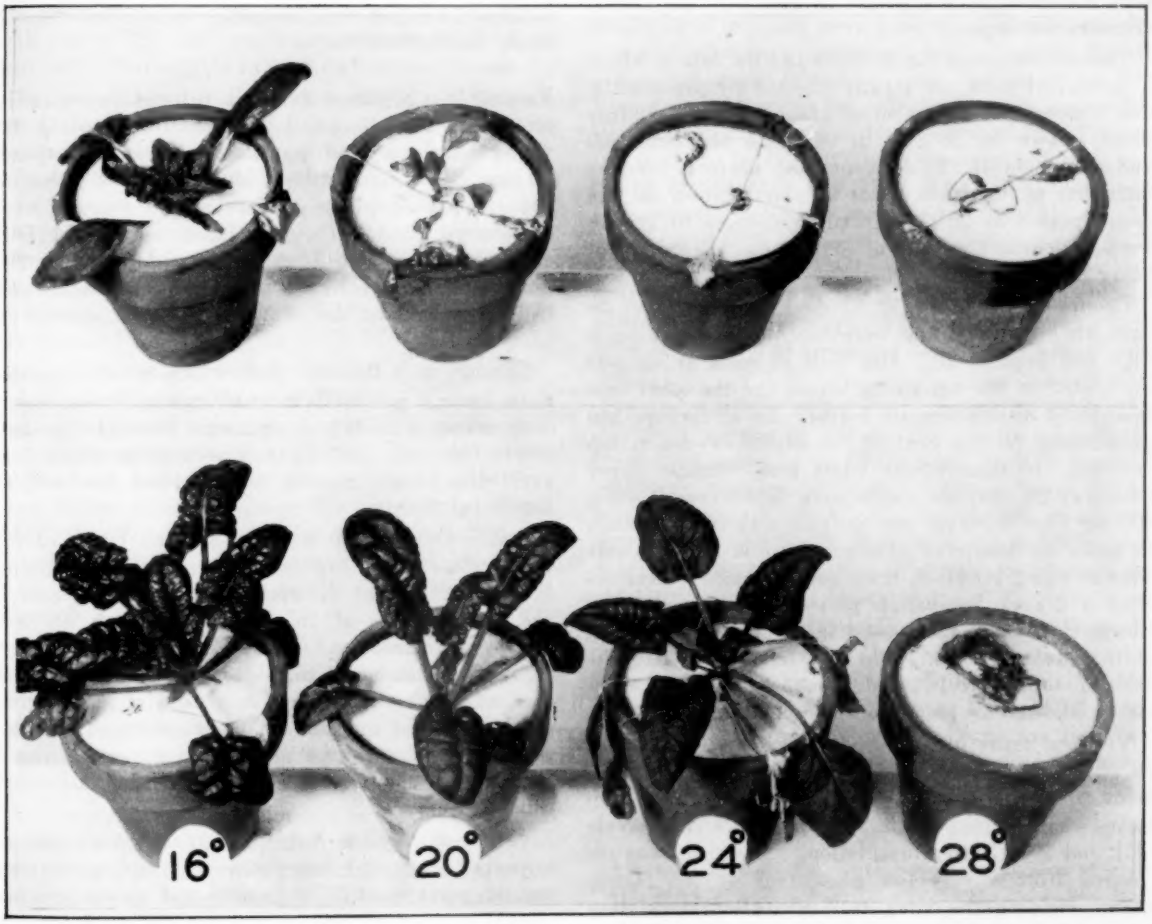


FIG. 1. Relative disease development in susceptible Nobel (top) and resistant Virginia Savoy (bottom) spinach when inoculated with cucumber virus 1 and kept for 25 days at 16°, 20°, 24°, 28° C.

With Virginia Savoy resistance was complete at 16° and 20° and no symptoms were evident. At 24°, very slight mottling and chlorosis with ultimate necrosis of lower leaves occurred but plants were never severely affected (Fig. 1). At 28° plants developed a necrosis of the apical meristem which extended downward, killing the plants within 7 days (Fig. 2). In 3 or 4 days after inoculation, small chlorotic spots were evident on inoculated leaves. These quickly became necrotic. About the fifth day after inoculation, necrosis, usually in a unilateral development, was evident in the youngest terminal leaves. After top necrosis appeared, young plants were completely necrotic in 24 hr. and old plants reached this condition in 48 hr.

Twenty-plant groups of Virginia Savoy plants growing at 16° in white quartz sand with nutrient solution were inoculated and exposed at 28° for 4 hr., 8 hr., 12 hr., 16 hr., 20 hr., and 24 hr. per day. After 30 days no necrosis had developed in any group kept at 28° for 12 hr. or less per day. Approximately 65 per cent of the plants of each of the groups exposed to 28° for 16 hr., 20 hr., and 24 hr. per day developed top necrosis. At the end of 30 days, all plants were kept continuously at 28° for 15 days. No additional necrosis developed.

The percentage of top necrosis and the rate at which it developed were not greatly affected by pre-inoculation temperature treatment of plants. Virginia Savoy plants grown for 30 days at 16° prior to inoculation and placement at 28° developed top necrosis one day later, but to the same extent as plants grown for the entire period at 28°. Marked differences in growth were evident at the time of inoculation, plants at 28° being taller and more spindly.

Groups of 56 plants at 22 days, 34 days, and 46 days after transplanting were inoculated at the same time and kept at 28°. One half of each group was inoculated on the top young leaves and the other half inoculated on the bottom leaves. In all groups approximately 65 per cent of the plants developed top necrosis. In none of the trials with Virginia Savoy plants at 28° did all plants show top necrosis, while 100 per cent infection was the rule with Nobel plants. In order to determine whether Virginia Savoy plants were escaping infection, those not showing top necrosis after a 2-week incubation period were reinoculated. Eleven days after the second inoculation 43 per cent of the plants surviving the first inoculation had developed top necrosis. Additional inoculations ultimately showed all plants to be susceptible.

Virginia Savoy plants resisted becoming infected at 16°. In several trials subinoculations to tobacco were made from inoculated leaves and from young top leaves of individual Virginia Savoy plants at intervals of 1 and 2 wk. after inoculation. The virus was recovered from a very low percentage of the plants. Plants yielding the virus at 2 wk. after inoculation often failed to do so in subinoculations at 30 days after inoculation. Thus it might be assumed that

FIG. 2. The top necrotic symptom on Virginia Savoy spinach inoculated with cucumber virus 1 and exposed to 28° C. air temperature

Virginia Savoy plants not only resisted becoming infected but also prevented the virus from moving into young top leaves, or that the virus was inactivated *in vivo*. This was further indicated by the failure of such inoculated plants to develop top necrosis when transferred to 28°. They developed top necrosis, however, if reinoculated after being transferred to 28°. Subinoculations at 7 days from inoculated Nobel control plants yielded the virus from 100 per cent of the plants tested.

Fulton[3] recently described a strain of cucumber virus 1 which was infectious to Virginia Savoy spinach. This strain was tested and was found to produce severe chlorosis, mottling and necrosis at all air temperatures, closely similar to symptoms produced on the Nobel variety.

SOIL TEMPERATURE RELATIONS.—The effect of soil temperature on the expression of resistance in Virginia Savoy spinach was determined by growing plants in soil temperatures of 16° and 28° at both 16° and 28° air temperatures. In one experiment all plants were grown under identical conditions until inoculation, at which time they were placed at the different combinations of soil and air temperatures. In another experiment plants were grown at the various air-soil temperature combinations for 2 wk. prior to inoculation.

In the susceptible Nobel variety, a severe necrosis appeared soon after inoculation in plants grown at a soil temperature of 28° irrespective of air temperature.

[3] Fulton, J. P. Studies on strains of cucumber virus 1 from spinach. Phytopath. 40: 729-736. 1950.

TABLE 1.—*The effect of air-soil temperature combinations on the percentage of plants showing top necrosis in Virginia Savoy spinach when inoculated with cucumber virus 1*

Days after inoculation	Percentage of plants showing top necrosis							
	Treated prior to inoculation [a]				Not treated prior to inoculation [b]			
	16° air temp.		28° air temp.		16° air temp.		28° air temp.	
	16° soil temp.	28° soil temp.	16° soil temp.	28° soil temp.	16° soil temp.	28° soil temp.	16° soil temp.	28° soil temp.
4	0	0	0	0	0	0	0	5
5	0	0	10	0	0	0	10	10
6	0	0	20	15	0	0	35	25
7	0	0	35	15	0	0	35	25
8	0	0	55	20	0	0	35	25
9	0	0	75	25	0	0	60	40
12	0	0	90	40	0	0	60	45

[a] Plants were grown for 2 wk. at respective temperature combinations prior to inoculation.
[b] Temperature treatments were started immediately after inoculation.

At the 28° air temperature this necrosis appeared on the bottom leaves in 6 days after inoculation and plants were killed within 16 days. Plants grown at 16° soil temperature and 28° air temperature developed little necrosis and plants were not killed until about 80 days after inoculation. At 16° air temperature and 28° soil temperature the necrotic reaction was identical with that at 28°—28° combination, except that plants were not killed until 3 to 4 wk. after inoculation.

Results obtained with resistant Virginia Savoy indicated that soil temperature did not determine the occurrence of top necrosis. Soil temperatures of 28° did not break down the resistance and produce top necrosis when the air temperature was kept at 16°. Also, a soil temperature of 16° did not reduce the amount of top necrosis when the air temperature was 28°, but on the contrary, tended to increase it, especially when plants were grown at this combination before inoculation (Table 1).

PHOTOPERIOD AND LIGHT INTENSITY RELATIONS.—Nobel and Virginia Savoy plants were grown under short day (8 hr.) and long day (16 hr.) exposures at air temperatures of 16°, 20°, 24°, and 28°. Day lengths were controlled by covering plants with black paper cages when the respective photoperiod terminated. Plants were grown directly beneath 200-watt incandescent lights which were controlled by a time clock. Treatments were begun 10 days prior to inoculation.

In the Nobel variety symptoms were more severe in short-day than in long-day plants, especially at 28°, plants being killed about 1 wk. earlier than in corresponding long-day plants. In Virginia Savoy plants top necrosis occurred only at 28° with little difference between long- and short-day plants.

One experiment was performed to see if light intensity influenced the development of top necrosis. Virginia Savoy plants were inoculated in the 4-leaf stage and one group was placed on an open greenhouse bench and another under a muslin cage. Incandescent lights of 200 watts were placed directly over each group. The relative light intensities were approximately 400 to 1600 foot-candles. Results obtained indicated that light intensity had no effect on the amount of top necrosis produced.

HOST NUTRITION RELATIONS.—Plants of resistant Virginia Savoy were grown in earthenware crocks in white quartz sand to which were applied solutions varying in levels of nitrogen, phosphorous, and potassium. Twenty plants for each treatment were inoculated at 28°. Plants were grown at the different levels being studied long enough prior to inoculation for marked differences in growth to be evident. With nitrogen, solutions contained 21, 210, 420, 630, 1050 parts per million; with phosphorous, solutions contained 3, 10, 31, 93, 237 p.p.m.; and with potassium, solutions contained 8, 78, 430, 704, 1410 p.p.m. All solutions were kept at identical osmotic values by the addition of sodium chloride where needed and, except for the element being varied, were at the level of Hoagland and Snyder's basic solution.[4]

In the nitrogen experiment, vegetative growth (as measured by wet or dry weight) increased with increased nitrogen up to 630 p.p.m. At 1050 p.p.m. a noticeable stunting occurred. A similar curve for top necrosis occurred (Table 2), the highest percentage occurring at 630 p.p.m. and the lowest at 1050 p.p.m. A relationship between the percentage of necrosis and vegetative growth was indicated.

In the phosphorus experiment, the growth response to the different levels of phosphorus was less marked than for nitrogen but a gradient increase was evident, beginning at 3 p.p.m. and increasing up to 93 p.p.m. after which it fell off. With phosphorus, too, there was a slight indication that top necrosis was related to vegetative growth, although differences were not great.

Growth responses to the different levels of potassium

[4] Hoagland, D. R. and W. C. Snyder. Nutrition of strawberry plant under controlled conditions: (A) effects of deficiencies of boron and certain other elements: (B) susceptibility to injury from sodium salts. Proc. Amer. Soc. Hort. Sci. 30: 228-294. 1933.

TABLE 2.—*The effect of different amounts of nitrogen, phosphorus, and potassium on the percentage of top necrosis induced in Virginia Savoy spinach by cucumber virus 1*

Days after inoculation	Percentage of plants developing top necrosis at concentration[a] level shown														
	Nitrogen					Phosphorus					Potassium				
	21	210	420	630	1050	3	10	31	93	237	8	78	430	704	1410
5	10	14	15	22	25	18	22	30	63	58	0	0	5	0	0
6	30	23	18	33	35	22	30	50	72	67	5	10	5	0	0
7	45	27	36	50	40	30	52	63	72	72	10	15	10	5	0
8	45	32	36	60	45	30	58	67	76	72	15	25	15	10	5
9	50	32	42	60	45	45	63	72	85	85	15	25	15	10	10
10	50	42	55	60	45	45	63	72	85	90	20	30	20	15	20
11	55	50	55	60	45	50	63	72	90	90	40	50	35	25	25
12	60	50	62	60	45	58	72	85	95	90	55	75	45	40	45
17	60	60	70	75	50	58	76	85	95	90	70	85	65	65	70

[a] Values shown refer to parts per million.

were slight. Correspondingly, differences in the amount of top necrosis were slight. At high concentrations, however, the appearance of top necrosis was delayed.

THE NATURE OF RESISTANCE.—To ascertain if resistance was related to virus concentration, comparable inocula from terminal leaves of susceptible Nobel and resistant Virginia Savoy plants inoculated and kept for 10 days, at 16°, 20°, 24°, and 28° were inoculated to the two unifoliate leaves of cowpea in opposite leaf comparisons. The 2 varieties were compared at each of the air temperatures. In the Nobel variety a temperature gradient of virus concentration (as measured by local lesions on cowpea) occurred, increasing from low to high temperature. From the Virginia Savoy plants practically no virus was recovered at any temperature. Many assays were made from Virginia Savoy plants with inoculum taken from the smallest leaves of top-necrotic plants and in all cases the virus concentration, as indicated by local lesion counts, was very low.

Inoculum from Virginia Savoy plants showing top necrosis was tested in a series of dilutions to see if an inhibitory substance was present which might be lost by dilutions low enough to leave the virus infectious. No appreciable increase in the number of local lesions was obtained at any dilution.

Since spinach plants have an inhibitory substance against cucumber virus 1 and other viruses[5], a test was made to see if there was any difference between susceptible and resistant plants in their inhibitory effect to cucumber virus 1. A standard inoculum was obtained from infected plants of *Nicotiana glutinosa* L., aliquots of which were treated with equivalent amounts of plant extracts from healthy Nobel and healthy Virginia Savoy spinach. Treatments were compared in all possible combinations in opposite leaf inoculations to cowpea. It was found that both Nobel and Virginia Savoy spinach extracts reduced the infectivity of the virus in the standard inoculum by about 99 per cent.

[5] Kuntz, J. E., and J. C. Walker. Virus inhibition by extracts from spinach. Phytopath. 37: 561-579. 1947.

Since high temperature increased the susceptibility of both susceptibile and resistant varieties to cucumber virus 1, it was deemed of interest to see if temperature had any effect on the inhibitory action of extracts from healthy plants. Extracts from healthy Virginia Savoy plants grown for 2 wk. at 16° and 28° were mixed with a standard inoculum obtained from infected tobacco plants. Inocula of equivalent dilutions were compared in all possible combinations on cowpea. Juice from Virginia Savoy plants grown at 16° and 28° each reduced activity of the virus in the standard inoculum by about 98 per cent.

Extracts from Nobel spinach, though highly inhibitory to inoculum taken from other plants, was less inhibitory to its own inhabiting virus as shown by the high lesion counts obtained from sap expressed from infected Nobel plants. This could mean that the virus multiplied in spinach at the expense of the inhibitor, that the multiplication of the virus resulted in neutralization of the inhibitor, or that the concentration reached was high enough to overshadow the effect of the inhibitor. To check on this point, extracts from infected Nobel plants and corresponding healthy ones grown at 24° were compared by adding separately to the same inoculum taken from tobacco. The following 4 inocula in equivalent dilutions were compared in all possible combinations: (1) 1 cc. of undiluted extract from infected tobacco plus 9 cc. of a neutral phosphate buffer; (2) 1 cc. of undiluted extract from infected Nobel spinach plus 9 cc. of buffer; (3) 1 cc. of undiluted extract from infected tobacco plus 1 cc. of undiluted extract from healthy Nobel spinach plus 8 cc. of buffer; (4) 1 cc. of undiluted extract from tobacco plus 1 cc. of undiluted extract from infected Nobel spinach plus 8 cc. of buffer. Results in Table 3 indicate that although the extract from infected Nobel plants contained a high virus concentration, it was highly inhibitory to the inoculum taken from tobacco, although slightly less so than the extract from healthy Nobel plants. Also, there was a mutual inhibition between the tobacco and spinach inocula. It may be concluded that the multiplication of virus in spinach plants did not coun-

TABLE 3.—*Comparative inhibitory effect of extracts from healthy and from infected plants of Nobel spinach to inoculum of the same virus taken from tobacco*

Opposite Leaf Comparisons[a]	Average number local lesions in 17 replications
IS	96[b]
IT	296
IT	272
IT + HS	2
IT	300
IT + IS	21
IT + HS	1
IT + IS	12
IS	136
IT + IS	14

[a] IS = extract from infected spinach; IT = extract from infected tobacco; HS = extract from healthy spinach. All preparations were in equivalent dilutions.

[b] Differences in each comparison were highly significant.

teract the inhibitory substance of extracts from such plants to the same virus from another source.

INHERITANCE OF RESISTANCE.—The F_1 and F_2 progenies of several crosses of Virginia Savoy × Nobel. Virginia Savoy × Viroflay and reciprocals, and Virginia Savoy × Prickly Winter were assayed in the greenhouse (Table 4). In each case the F_1 was completely resistant and segregation in the F_2 approximated 3 resistant to 1 susceptible. Similar data were obtained in crosses with Old Dominion, indicating that resistance of this variety was the same as for Virginia Savoy. It appears that resistance is controlled by a single dominant gene.

TABLE 4.—*Inheritance of resistance to cucumber virus 1 in spinach*

Progeny	Generation	No. Plants Inoculated	Segregation Res.	Suscept.	Theoretical 3:1 Ratio in F_2
2515-2[a]	F_1	50	50	0	
2515-2M	F_2	75	56	19	56:19
2515-3	F_1	30	30	0	
2515-3M	F_2	104	76	28	78:26
2015-5	F_1	30	30	0	
2015-5M	F_2	117	86	31	88:29
2015-6	F_1	30	30	0	
2015-6M	F_2	164	125	39	123:41
2015-7	F_1	30	30	0	
2015-7M	F_2	220	169	51	165:55
2015-8	F_1	30	30	0	
2015-8M	F_2	190	142	48	143:47
1520-2	F_1	30	30	0	
1520-2M	F_2	211	149	62	158:53
3015-1	F_1	30	30	0	
3015-1M	F_2	59	39	20	44:15
3015-2	F_1	30	30	0	
3015-2M	F_2	47	35	12	35:12

[a] 2515 = Nobel × Virginia Savoy; 2015 = Viroflay × Virginia Savoy; 1520 = Virginia Savoy × Viroflay; 3015 = Prickly Winter × Virginia Savoy.

SUMMARY AND CONCLUSIONS

Air temperature had a marked effect on development of symptoms and on disease severity in both susceptible and resistant varieties of spinach inoculated with cucumber virus 1. In the susceptible variety Nobel, high air temperature not only shortened the incubation period, but also increased the killing effect of the virus. Infected plants were killed in about 80, 28, 22, and 16 days at 16°, 20°, 24°, 28°, respectively, when soil temperatures were about the same as air temperatures. This lethal effect, due chiefly to necrosis, was accentuated by high soil temperature. Infected plants grown at 16° air temperature and 28° soil temperature were killed in 25 to 27 days rather than 80 days when at 16° air and 16° soil temperature. Exposing plants to the 16°–28° air-soil temperature combination for 2 wk. prior to inoculation resulted in death in 18 to 20 days after inoculation. On the other hand, infected plants kept at an air temperature of 28° and a soil temperature of 16° died in about 26 days while those at 28°–28° combination died in about 16 days after inoculation. Short day length (8 hr.) and low light intensity (approximately 400 foot-candles), both of which retarded growth of spinach, induced more severe symptoms and earlier death than long day length (16 hr.) and high light intensity (approximately 1600 foot-candles).

In the resistant variety, Virginia Savoy, resistance was expressed at air temperatures of 16° and 20°, although plants were not always immune to infection. However, they resisted infection at these temperatures and only rarely were plants infected. At 28° this resistance broke down and plants succumbed to a systemic necrosis which began in the apical meristem. A 16-hr. exposure per day at 28° was required for the production of this top necrosis. Infected plants kept at 24° showed slight mottling but no further ill effect.

High soil temperature was not responsible for the production of top necrosis in resistant plants. Inoculated plants at 28°–16° air- soil temperatures developed even more top necrosis than plants kept at the 28°–28° combination while plants kept at a 16°–28° air-soil combination showed complete resistance.

Other environal factors such as day length, light intensity and host nutrition had little influence on the production of top necrosis in resistant plants at 28° air temperature.

At all air temperatures studied, infection could be obtained in resistant Virginia Savoy plants but the latter resisted becoming infected at temperatures below 28°. In the few plants infected at 16° and 20° the virus must have been inactivated in the host or else further multiplication was inhibited, for when those plants were moved to 28° no top necrosis developed and the virus was rarely recovered by subinoculations.

Moreover, the virus could be recovered by subinoculations to tobacco soon after inoculation at 16° but not after about 15 days. However, virus multiplication in resistant plants was not detectable, even from plants inoculated at 28°, by the local-lesion assay on cowpea.

The inhibitory substance present in all spinach plants did not inhibit the virus present in Nobel plants themselves when the tissue was macerated, but did inhibit the same virus in inoculum from some other plant. The multiplication of cucumber virus 1 in spinach does not destroy this inhibitor, since infected spinach extract produced approximately the same inhibitory effect to inoculum from tobacco as did healthy spinach extract. Extracts from susceptible and resistant spinach plants did not differ in their inhibitory effects to cucumber virus 1 in inoculum taken from tobacco.

Assays of progenies of crosses of resistant Virginia Savoy × susceptible varieties indicated that resistance is controlled by a single dominant gene.

Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

RELATION OF AIR TEMPERATURE, SOIL TEMPERATURE, PHOTOPERIOD, AND LIGHT INTENSITY TO THE CONCENTRATION OF CUCUMBER VIRUS 1 IN SPINACH[1]

Pen-Ching Cheo and Glenn S. Pound

Air temperature markedly influences the development of cucumber virus 1 in both resistant and susceptible varieties of spinach (8). Temperatures of 28° C. or above are lethal to all virus-infected plants while low temperatures retard disease development in susceptible plants and inhibit symptom expression in a resistant variety. This paper deals with the question of relationship between these effects and virus concentration.[2] It is also concerned with effects produced by other environal factors such as soil temperature, day length, and light intensity, since they may be directly related to the apparent effects of air temperature.

Materials and methods.—Plants of the susceptible Nobel variety of spinach were grown singly in 4 in. pots in greenhouse compost mixed with sand. They were inoculated with sap taken from *Nicotiana glutinosa* L.

Assay samples for virus concentration consisted of 1 g. of tissue from the youngest leaves from 10 or more plants from each treatment macerated in 6 cc. of a neutral phosphate buffer.

Cowpea (*Vigna sinensis* Endl. var. Black) plants used as an assay host were predisposed by keeping them in light-free cages for 24 hr. prior to inoculation, as suggested by Sill and Walker (11). Opposite leaf comparisons were made on the unifoliate leaves. Statistical analysis of uniformity data had revealed no difference between the 2 halves of a leaf or between the opposite unifoliate leaves of a plant. Finely powdered aluminum silicate was used as an abrasive, and inocula were applied with glass spatulas. Plants were rinsed with distilled water immediately after inoculation.

The strain of cucumber virus 1 used was isolated from mosaic-infected spinach near Racine, Wisconsin. Its identity was proved by cross protection tests with Price's No. 6 indicator strain (9).

Experimental results.—*Effect of air temperature.*—Spinach plants grown at 22° C., were divided after inoculation among 4 air temperatures (16°, 20°, 24°, 28° C.), 12 plants at each temperature. At about 3-day intervals, for 5 or 6 assays, inocula taken from the youngest systemically-infected leaves of plants at the 4 different temperatures were compared in all possible combinations. For each opposite leaf comparison, at least 16 cowpea plants were used, giving at least 16 replications for each comparison and 48 replicates of each inoculum.

In several trials there was a definite cycle of virus activity at each temperature (Fig. 1). For about the first 7 days after inoculation plants at 28° showed the highest virus concentration followed in order by plants at 24°, 20°, and 16°. After 7 days, virus concentration at 28° apparently declined. At the same time, the concentration at other temperatures increased and at the 10-day assay it was highest at 24°, followed in order by 20°, 28°, and 16°. Thus, 10 days after inoculation, the peak concentration had been reached in plants at 28° and 24° after which it steadily decreased. On the other hand, the concentration at 20° and 16° steadily increased. About 12 days after inoculation the concentration was highest at 20°, followed in order by 16°, 24°, and 28°. Assays after 12 days showed the highest concentration occurred in plants at 16°.

Virus concentration did not appear to be correlated directly with symptom severity. Plants at 16° developed symptoms slowly. Virus concentration was sufficient after 7 days to produce only a few local lesions on cowpea. The concentration steadily increased for several days and then gradually declined even though symptoms became progressively more severe until death occurred in about 80 days. In one trial, the concentration in plants kept at 16° for 21 days was

[1] Accepted for publication February 29, 1952.
Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] The term virus concentration in this paper refers to the virus activity in plant extracts as measured by local lesion counts.

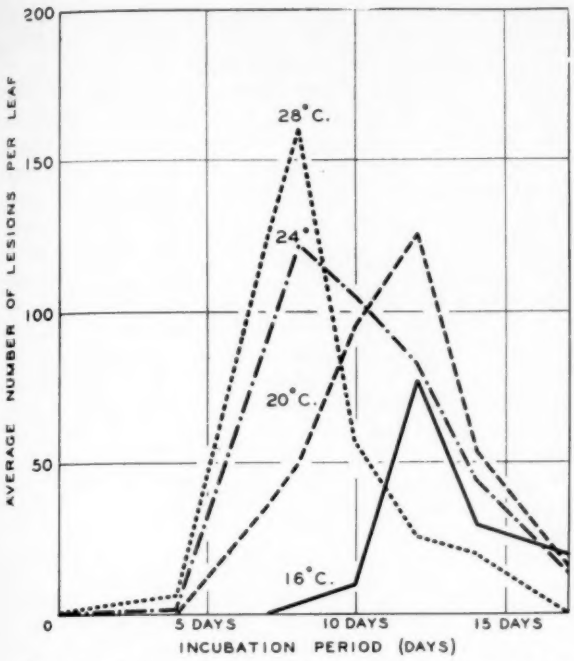


FIG. 1.—Effect of air temperature on the concentration of cucumber virus 1 in spinach at different time intervals after inoculation.

compared with that in plants kept at 16° for 65 days. Average numbers of local lesions per leaf were 14.4 and 0.5, respectively. In another trial, plants kept at 16° for 28 days were divided into 2 groups, 1 group was moved to 28° while the other was kept at 16°. Subsequent assays showed no increase in concentration at 28°. Thus, after symptom severity reaches a certain stage, growth is so inhibited that virus multiplication is greatly reduced.

In one experiment plants were grown at temperatures of 16°, 20°, 24° and 28° for more than a month prior to inoculation. A marked gradient of growth, inversely proportional to the air temperature, was evident at the time of inoculation. This experiment yielded results very similar to those shown in Fig. 1.

Since cucumber virus 1 is lethal to spinach, it was thought probable that the virus cycle within the spinach plants was influenced by the declining vigor of the host in the latter phase of the cycle. At the beginning the rate of virus multiplication was favored by higher temperatures, but, later, when virus accumulated to a certain maximal amount, the direct injurious effect of the virus to the host caused gradual death of the plant. This would explain the decline phase of the virus cycle within the host plant, regardless of temperature. To check this point, tobacco was similarly studied at the 4 temperatures. Tobacco was chosen because it is a high temperature plant, whereas spinach is a low temperature plant, and infection with cucumber virus 1 is not lethal to tobacco at any temperature being studied.

Essentially the same cyclic activity as described for spinach was found in tobacco. However, at each temperature infected tobacco plants showed periods of marked symptom expression followed by periods of recovery from symptoms with corresponding periods of high and low virus concentration. The frequency of these cycles depended upon the temperature (Table 1).

Effect of soil temperature.—At 16° and 28° air temperatures, spinach plants were grown in sand in soil temperature tanks kept at 16° and 28°. Prior to inoculation they were all kept at the same air and soil temperatures. Inoculations were made at the 4-leaf stage at which time the plants were distributed to the different soil temperature tanks. Assays on cowpea were made at 3-day intervals from plants at 28° air temperature and at 7-day intervals from plants at 16° air temperature.

TABLE 1.—*The effect of air temperature on the concentration of cucumber virus 1 in tobacco*

Temperature Comparisons[a]	Average number local lesions at intervals (days) after inoculation						
	4[b]	8	12	17	23	29	36
16°	0.0	7.0	114.2	263.2	314.3	94.6	29.7
20°	0.0	144.5**	209.6**	337.1**	17.9**	7.1**	90.4**
16°	0.0	9.1	94.5	251.8	269.1	102.6	37.8
24°	[c]	262.9**	170.3**	11.8**	33.3**	70.2**	29.9
16°	0.0	7.4	113.4	242.7	398.3	135.3	42.5
28°	[c]	280.9**	72.1**	113.3**	312.1**	88.1**	75.0**
20°	0.0	163.8	156.4	272.8	17.7	8.3	56.1
24°	[c]	189.4	180.9	10.6**	46.5**	69.9**	17.4**
20°	0.0	229.5	151.2	281.3	16.4	9.6	81.7
28°	[c]	305.2**	54.6**	102.0**	280.2**	66.8**	62.3
24°	395.7	226.0	178.1	12.6	52.7	98.3	25.4
28°	458.3*	250.2	84.3**	127.6**	306.5**	58.3**	78.7**

[a] Each comparison made on a separate group of assay plants.
[b] Inocula at 4 day assay were diluted 1 to 10. In all other assays dilutions were 1 to 20.
[c] Lesions not counted.
* Differences between temperature comparisons significant at the 5 per cent level.
** Differences between temperature comparisons significant at the 1 per cent level.

Virus concentration was increased by high soil temperature with host vigor as a limiting factor. At 28° air temperature, virus concentration was higher at 28° soil temperature than at 16° soil temperature up to 6 or 8 days after inoculation. After that, higher virus concentration occurred at the 16° soil temperature. It is believed that the drop in virus concentration at 28° soil temperature was due to a severe necrosis which developed about that time.

At 16° air temperature, the effect of high soil temperature on virus concentration was more marked than at 28° air temperature. The virus concentration was much higher in 28° soil than in 16° soil for about 18 days. About this time, necrosis of plants in the 28° soil became predominant and virus concentration in subsequent assays fell below that in 16° soil.

A similar experiment was performed in which plants were grown at the different combinations of soil and air temperatures for 2 wk. prior to inoculation. At the end of the preinoculation treatment slightly better growth and color were evident in plants in 16° soil temperature, especially at 28° air temperature. Results of this experiment coincided closely with the one in which plants received no preinoculation treatment. Higher concentration occurred in plants in 28° soil than in 16° soil at both air temperatures, for a few days. Later, following the development of necrosis in 28° soil, the concentration was higher in the 16° soil.

It may be concluded that cucumber virus 1 in spinach is favored by high soil temperature as long as the host vigor is good. However, the effect of soil temperature on virus concentration was less marked than the effect of air temperature.

The effect of photoperiod and light intensity.—Day length and light intensity markedly affect growth and bolting of spinach plants. Bolting is hastened by long days. Day lengths were controlled by covering the plants with black paper cages when the respective day length terminated. Plants were grown beneath 200-watt incandescent lights which were controlled by a clock set for 16 hr. illumination (6 A.M. to 10 P.M.). The air temperature was kept at 26° C.

In 1 experiment plants were grown in the different day lengths (8 hr. and 16 hr.) for 3 wk. prior to inoculation at which time marked growth differences were evident. After inoculation photoperiod treatments were continued to the end of the experiment. In general, short-day plants were dwarfed and rosetted without bolting while long-day plants were tall, had more foliage and readily bolted. In 3 other experiments day-length treatments were begun only 1 day prior to inoculation but were continued to the end of the experiment. Thus, no differences in vegetative growth occurred prior to inoculation. In all of these tests (Table 2), long-day plants showed a much higher virus concentration than short-day plants. This was true throughout the whole disease cycle. This is different from the air temperature relation where the virus concentration was highest only at certain stages of the disease cycle.

Two experiments were performed in which photoperiod treatments were begun 1 day after inoculation. This test differed from the others only in that at 4 to 5 days short-day plants showed the highest virus concentration. From that time on, long-day plants showed significantly higher concentration than short-day plants (Table 2). No explanation is at hand for the higher virus concentration in short-day plants for the first few days after inoculation. It may be that longer periods of darkness favored movement of the virus; movement during the early stages of incubation was greater in short-day plants. When plants were exposed to different day lengths prior to inoculation the physiology of long-day plants could have so favored multiplication of the virus that more rapid movement in short-day plants was not detectable.

To see what effect on the virus concentration differences in vegetative growth caused by previous exposure

TABLE 2.—*Effect of photoperiod on the concentration of cucumber virus 1 in Nobel spinach*

Photoperiod treatment	Days after inoculation	Av. no. lesions per leaf		F Value
		8 hr. day	16 hr. day	
Begun 14 days prior to inoculation and continued throughout experiment	4	7.3	37.8	36.9**
	8	72.8	187.6	30.5**
	10	41.2	86.7	52.2**
	14	14.4	26.5	22.9**
Begun one day prior to inoculation and continued throughout experiment	3	1.3	3.0	7.7*
	6	159.0	364.8	79.5**
	9	73.6	187.0	30.3**
	12	24.8	43.5	9.9**
	15	13.4	54.8	23.4**
Begun one day after inoculation and continued throughout experiment	5	43.6	8.4	34.5**
	7	13.4	54.9	80.7**
	9	17.9	75.7	104.8**
	12	19.1	34.8	22.4**
	14	3.1	12.6	29.8**

* Significant at 5 per cent level.
** Significant at 1 per cent level.

to long and short days might have had, plants were kept at the different day lengths for 3 wk. prior to inoculation. After inoculation all plants were set on an open bench and exposed only to ordinary day length. Results obtained indicated that the difference in vegetative growth caused by the different day lengths was not responsible for the higher concentration in long-day plants. Plants previously exposed to short days showed a higher virus concentration than long day plants up to 10 days after inoculation. After that, the reverse was true. Short-day plants were dwarfed and physiologically young at the time of inoculation while long-day plants were taller and physiologically older. Thus after inoculation and termination of day length treatments, the difference between these 2 sets of plants was no longer a matter of day length but of age. This was proved by experiments in which 2 sets of plants of different ages were compared. Young plants were in the 4 or 5-leaf stage, while the old plants were bolting. These growth differences were very similar to growth conditions of plants exposed for 3 wk. prior to inoculation at short and long days, respectively. They were grown under the same day length and at the same temperature before and after inoculation. Assays showed virus concentration to be higher in young plants up to 11 days. Afterwards, due to the severity of the disease, the young plants rapidly declined, and concentration was lower than in old plants which had still retained most of their vigor.

Since difference in growth was not the cause of the high virus concentration in spinach plants under a long day, it was of interest to see if intensity of light would have the same effect as duration of light. Spinach plants were grown under 200 watt incandescent lights at 16 hr. illumination. One half the plants were covered with a muslin cage while the other half were left uncovered. The muslin cage reduced the light intensity from approximately 1600 ft. candles to approximately 400 ft. candles. In 2 different experiments, assays indicated that the high light intensity increased the virus concentration both in plants treated for 14 days prior to inoculation and in plants where treatments were begun 1 day after inoculation. However, this difference cannot be attributed entirely to differences in light intensity since the temperature inside the muslin cage was 2° to 3° lower than that outside. It is believed, however, that the difference was primarily due to light intensity, since in each of 4 successive assays, the concentration under high light intensity was greater than under low intensity. If the difference had been due to temperature, a reversal in concentration would have been expected by the time of the fourth assay.

SUMMARY AND CONCLUSIONS

Using a local lesion host to test the comparative virus infectivity of sap extracts of spinach plants infected with cucumber virus 1 and kept at different air temperatures, it was found that within each temperature there was a definite cycle of virus activity. At 28°, the virus content increased from about the second to the eighth day after inoculation, then decreased rapidly until death occurred on or about the sixteenth day. At 24°, the virus content gradually increased until about the eleventh day after inoculation and then decreased until death at about 22 days. At 20°, the virus content of infected plants steadily increased to the thirteenth day after inoculation and then gradually decreased until death occurred at about 30 days. At 16°, the virus concentration increased steadily to the fifteenth day after inoculation and then leveled off. Plants at 16° were not killed until 80 days after inoculation but the virus concentration after 30 days was very low.

It cannot be concluded from these studies that an air temperature of 28° ultimately increased virus concentration over that at 24° and 20°. The studies showed that 8 days after inoculation the highest virus concentration was from plants at 28°; at 10 days after inoculation at 24°; at 12 to 14 days after inoculation at 20°; at 17 days after inoculation at 16°. Thus, the highest virus concentration obtained from the 4 temperatures depended upon the time interval after inoculation. The temperature affected the rate of the virus cycle more than the virus content. However, the number of lesions obtained at the peak of the cycle from plants at 16° was always lower than that obtained from other temperatures at their respective peaks.

The same virus cycle was also observed in tobacco plants infected with cucumber virus 1. About 4 to 8 days after inoculation, tobacco plants at 28° showed the highest virus concentration. At about 12 days the highest virus concentration was in plants grown at 24°; at 17 days from plants at 20°; and at 23 to 29 days, from plants at 16°. The number of lesions obtained from plants at 16° at their peak concentration was just as great as the highest number obtained from 28°. Following the peak at each temperature there was a rapid decline in concentration. This coincided closely with partial recovery from symptoms. Following the recovery period, plants showed a recurrence of symptoms which coincided with secondary peaks by virus concentration. The interval of time between these cycles was affected by temperature. Old leaves of tobacco plants showed less and slower recovery from symptoms than young leaves. Inoculated leaves might have shown a different picture of virus concentration had they been assayed.

In spinach, the decrease of virus after a maximum peak was not accompanied by recovery from symptoms. In plants kept at 16° the virus concentration declined after the seventeenth day but symptoms became progressively more severe for some 60 days. Infected plants made very little additional growth. The rapid decrease in the virus concentration after 17 days at 16° might have been due to a point of saturation after a maximum multiplication of the virus. Tobacco plants, however, continued to produce new growth. The virus multiplication after reaching a maximum could become static at which time newly-formed

leaves would show few symptoms. Thus, it would take another incubation period for maximum multiplication to occur in this area. This would later reach another peak and would be followed in turn by another period of symptom recovery and low virus concentration. This cyclic activity would occur as long as plants continued to produce new growth.

The cyclic activity at different temperatures is different from temperature effects of certain crucifer viruses reported by Pound & Walker (5, 6) and Pound (7). In these hosts, when certain temperatures effected recovery, it was permanent as long as that temperature prevailed. Virus concentration was generally correlated with symptom severity. With host vigor as a limiting factor, virus concentration in spinach plants grown at 28° soil temperature was greater than that in plants grown at 16° soil temperature.

Longer day length and high light intensity favored multiplication of cucumber virus 1 in spinach. These effects were not due to differences in growth resulting from different treatments but rather to the direct effect of longer duration and higher intensity of light. Best reported that more local lesions were produced by the tomato spotted wilt virus on tobacco plants kept in the shade than on plants in open sunlight (3, 4). Bawden and Roberts (1, 2), using tobacco necrosis, tomato bushy stunt, aucuba mosaic, and tobacco mosaic viruses, reported that shading plants prior to inoculation increased their susceptibility to a local lesion reaction as well as the total virus content attained in systemically-infected leaves. This effect on systemic development is contrary to the results with cucumber virus 1 reported here. In the present study, however, the effect of light intensity was not completely separated from a temperature effect.

Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

LITERATURE CITED

1. Bawden, F. C., and F. M. Roberts. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. **35**: 286-296. 1948.
2. ———, and ———. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418-428. 1948.
3. Best, Rupert J. The effect of environment on the production of primary lesions by plant viruses. Jour. Austr. Inst. Agri. Sci. **1**: 159-161. 1935.
4. ———. The effect of light and temperature on the development of primary lesions of the virus of tomato spotted wilt and tobacco mosaic. Austr. Jour. Exp. Biol. and Med. Sci. **14**: 223-239. 1936.
5. Pound, G. S. The effect of air temperature on virus concentration and leaf morphology of mosaic-infected horseradish. Jour. Agr. Res. [U.S.] **78**: 161-170. 1949.
6. ———, and J. C. Walker. Differentiation of certain crucifer viruses by use of temperature and host immunity reactions. Jour. Agr. Res. [U.S.] **71**: 255-278. 1945.
7. ———, and ———. Effect of air temperature on the concentration of certain viruses in cabbage. Jour. Agr. Res. [U.S.] **71**: 471-485. 1946.
8. ———, and Pen-Ching Cheo. Studies on resistance to cucumber virus 1 in spinach. Phytopath. (Abstr.) **42**: 18. 1952.
9. Price, W. C. Acquired immunity from cucumber mosaic in zinnia. Phytopath. **25**: 776-789. 1935.
10. Sill, W. H. Jr. and J. C. Walker. Cowpea as an assay host for cucumber virus 1. Phytopath. **42**: 328-330. 1952.

ROOT INJURY AS A FACTOR IN THE ASSESSMENT OF CHEMOTHERAPEUTANTS [1]

W. G. Keyworth [2] and A. E. Dimond [3]

In assaying chemotherapeutants by means of the Fusarium wilt test (5,7), the writers noted that certain compounds which reduced disease severity also caused injury to the roots of test plants. Notable among such compounds was n-octadecyltrimethylammoniumpentachlorophenate (4), hereafter referred to as HD 3. This injury usually consisted of a considerable reduction in root size, accompanied by cortical browning and occasional complete necrosis of the root system. The present study was undertaken to determine whether the reduction in disease was in any way related to root injury.

The procedure in the Fusarium wilt assay involves applying the test compounds to the roots of plants over a period of 10 days before inoculation. Each treatment is applied to 5 plants, arranged on the greenhouse bench in a randomized block. These roots are then inoculated by dipping in a bud-cell suspension of *Fusarium lycopersici*. The effectiveness of the compounds is assessed by subsequent wilting.

In recording wilt symptoms, extent and severity of leaf wilting and extent and intensity of vascular discoloration were considered. The bottom 3 leaves were disregarded, since some are shed naturally. The remaining ones were graded individually, 0 indicating a healthy leaf and 4 one that was dead or missing. The sums of the grades were divided by the number of recorded leaves to give an average for each plant. The maximum possible reading was thus 4.0. Vascular discoloration was noted at each internode, each of the 6 vascular bundles being rated from 0—3 for intensity of browning. The sums of the internode readings were divided by the number of internodes, multiplied by 6 (the number of bundles in the stem). The maximum vascular grade obtainable was 3.0. Average wilt grades were computed by both methods of grading for each treatment.

EXPERIMENTS ON ROOT INJURY.—To test the effect of root injury on subsequent disease expression, roots of tomato plants were injured before inoculation by (1) dipping in hot water immediately before inoculation, (2) periodic pruning, or (3) exposing to compounds which damage the roots but do not cause necrosis of aerial parts.

Hot water treatments immediately before inoculation.—To injure the roots of tomatoes without injuring aerial parts of the plant, immersion of roots for 30 sec. at a temperature of 55°C. was used. The treated roots died within 3 days and during this time many adventitious roots started to grow from the base of the stem. When plants were inoculated with *Fusarium*, bud cells were first centrifuged and washed to eliminate phytotoxic components in the culture filtrate.

It was necessary to carry several types of check in this experiment, since vascular discoloration was used to distinguish among treatment effects. In addition to the plants receiving hot water treatment plus inoculation with *Fusarium* (HWF plants), another lot of plants was subjected to hot water without inoculation (HW). Another lot was inoculated without hot water treatment (F), while a fourth lot received no treatment (C). Since the detailed pattern of vascular discoloration in plants was of particular interest in this experiment, readings were taken at each inch of stem length, rather than at each internode. The response of plants 21 days after inoculation is shown in table 1.

TABLE 1.—*Effect of treating roots with hot water upon their susceptibility to Fusarium wilt*

Treatment (see text)	Av. grade of vascular discoloration
F	1.60
HWF	0.36
HW	0.02
C	0.00

There was a marked decrease in susceptibility of tomato plants to Fusarium wilt when their roots were injured by soaking in hot water just before they were inoculated. These data suggest that compounds which cause the roots to die shortly after inoculation may have the same effect.

Periodic root pruning.—The object of the root pruning experiments was to subject roots to a continuing injury over a period of 10 days before inoculation and thus simulate the effects of root injuring compounds. The effects of a single drastic pruning immediately before inoculation were first tested. This showed that plants whose roots were cut to $\frac{3}{8}$ in. just before being inoculated developed severe disease. For periodic pruning the plants were grown in wire mesh baskets, uprooted 9, 5, and 2 days before inoculation, the roots cut back to the level of the basket and then replanted. Plants with uncut roots, with and without wire baskets, constituted checks. Root pruning stunted the treated plants to about $\frac{2}{3}$ normal size and made them darker green. Just before inoculation, the tips of the roots of all the plants were broken off to provide wound entry points for the fungus.

Results demonstrated that the only significant difference in disease severity between the plants resulted

[1] Accepted for publication February 28, 1952.
The authors wish to express their appreciation of the considerable help of Miss Barabara Wooding throughout the whole of this investigation. They are also indebted to Messrs. R. A. Botsford, L. G. Kierstead, and W. T. Mathis for the chemical analyses of plant constituents.

[2] Plant Pathologist, East Malling Research Station, Kent, England, stationed at The Connecticut Agricultural Experiment Station from November, 1950 to November, 1951.
[3] Chief, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station.

TABLE 2.—*Wilt records on plants subjected to periodic root pruning*

Treatment	Number of plants	Av. leaf grade	Av. vascular grade
Roots pruned	15	1.40	0.23
Roots normal	20	2.57	0.97

from the cutting treatment and that the presence or size of the baskets did not influence the degree of wilting (Table 2). Plants subjected to periodic root pruning showed a very great reduction in disease severity.

In a duplicate experiment on periodic pruning of roots, the reduction in disease was much less marked, vascular grades being 0.70 for treated plants and 1.04 for checks. A third trial confirmed the results of the second; apparently the striking differences obtained in the first experiment cannot be repeated readily. In all experiments plants with periodically pruned roots were less severely attacked by *Fusarium* than plants with normal roots, and in all cases care was taken to provide many found entry points for the fungus. Since the fungus could probably enter through pruned roots, the eventual suppression of disease must be attributed to an increase in the resistance of the plants.

Chemical root injury.—a) *Tests of root damaging compounds.* The third method of inducing root injury was to apply a series of compounds selected solely for their ability to injure roots without grossly injuring aerial parts of the plants. Nine compounds were selected as fulfilling the necessary requirements: sodium sulfate, potassium bromide, formalin, hydrogen peroxide, and the chlorides of aluminum, calcium, ferric iron, potassium and sodium. These were applied according to the normal schedule for the chemotherapy assay, and all except $FeCl_3$ were applied in dosage series to determine the concentration at which root injury was greatest without injury to aerial portions of the plant and the response of inoculated plants to these dosages. These studies showed that where root injury was high the severity of Fusarium wilt was low. Pertinent data are included in Table 3, in which wilt grades of the treated plots are expressed as percentages of the check. It will be noted that all compounds caused a marked reduction in disease severity.

In general the greatest amount of root injury was associated with the greatest extent of disease reduction. These compounds had only one feature in common, viz. the production of root injury. These facts suggest that disease reduction was not related to the specific properties of any individual compound but rather to their root-injuring capacity.

Compounds at dosages which caused stunting and browning only are of greatest interest since this is also the effect of HD 3. Hydrogen peroxide, outstanding in this respect, was selected for further experiments designed to study more closely the possible mechanism of disease reduction.

b) *Removal of chemically injured roots before inoculation.* Even though roots were broken off just before inoculation to provide entry wounds for the pathogen, the fungus may gain entry through unbroken root surfaces, since these were bathed in bud suspension. Superficial chemical injury of the roots might thus influence the ease of invasion by *Fusarium*.

To examine this possibility, roots of check plants and those treated with various concentrations of HD 3 and hydrogen peroxide were cut back to about $\frac{3}{8}$ in. just before inoculation. As noted previously such cutting of the roots immediately before inoculation did not affect the severity of wilt in normal plants (Table 4).

TABLE 3.—*Effect of root-damaging compounds on the severity of Fusarium wilt*

Compound	Conc. pct.	Appearance of roots	Wilt grades (% check) Leaf	Vascular
$Al\ Cl_3$	1.0	Stunted and brown	27	22
Formalin	0.08	Slightly stunted and brown	42	46
NaCl	4.0	Severely rotted	20	11
$CaCl_2$	3.0	Greatly stunted, brown and somewhat rotted	16	3
$FeCl_3$	0.5	Severely rotted	22	14[a]
Na_2SO_4	5.0	Stunted and brown, slightly rotted	41	0
KCl	2.0	Greatly stunted and brown	16	3
KBr	3.0	Stunted and brown	41	0
H_2O_2	0.6	Greatly stunted and brown	3	0
HD 3	0.005	Stunted and brown	16	1
Check	——	Normal	100	100

[a] Discoloration very intense. Probably caused by compound.

TABLE 4.—*Effect on wilt severity of removal of chemically injured roots before inoculation*

Treatment	Conc. %	Root Cutting	Leaf Index	Vasc. Index
HD 3	.005	—	.34	.01
	.005	+	.36	.06
H_2O_2	.6	—	.07	0
	.6	+	.07	0
	.8	—	.17	0
	.8	+	.04	0
Check	—	—	2.08	1.08
	—	+	1.76	.92

Data in Table 4 indicate that even though the bulk of the roots was removed and many wound entry points thus exposed near the stem, the plants treated with HD 3 and hydrogen peroxide still showed little or no disease. Thus, inhibition of the pathogen at the root surfaces was not responsible for reduction in disease. The fungus must have been checked in either the upper parts of the roots or in the lower stems of the plants.

It seems reasonable to regard the 2 compounds used in this experiment as typical of the root damaging compounds as a whole. The effects observed were presumably caused by the root injury alone and not by any specific action of the compounds; if this is so, then prolonged root injury before inoculation makes the tomato plant resistant to extensive invasion by *Fusarium*.

c) *Analysis of treated plants.* Davis (6) has reported that tomato plants treated with 4 chloro 3,5 dimethylphenoxyethanol (1182) contain more reducing sugars (or other reducing substances) than do normal plants. An experiment was therefore conducted to investigate the effect of treatment with 1182, HD 3 and hydrogen peroxide on certain chemical constituents of tomato plants. Plants were treated with these compounds by the standard method so that comparable groups could be withdrawn for analysis on successive days.

Two such groups of 5 plants were taken from each treatment on the tenth and eleventh days after treatment was started. Lateral roots were cut from all the plants and each group weighed. The plants were cut up, covered with water, and macerated in a Waring Blendor for 2 min. The resultant plant pulp mixture was filtered and the filtrate made up to a standard volume. Aliquots were subjected to the standard analysis for reducing substances using Fehlings solution (1). A duplicate aliquot was analysed after acid hydrolysis. The results of these analyses are given in Table 5.

TABLE 5.—*Cu_2O equivalents of water-soluble reducing substances present in treated and untreated tomatoes*

Treatment	Sample[a]	Av. weight of plants (gms.)	Av. Cu_2O equiv. (mgm) per gm. plant weight	
			Before hydrolysis	After hydrolysis
1182 (.003%)	1	9.2	13.8	14.0
	2	9.2	11.9	12.5
HD 3 (.005%)	3	8.6	14.3	14.8
	4	9.6	13.1	13.7
H_2O_2 (.6%)	5	9.4	9.8	9.9
	6	9.0	10.3	10.6
Check	7	16.6	5.3	5.9
	8	18.8	4.9	4.9

[a] Plants with odd numbers were harvested on the tenth day and even numbers on the eleventh day.

Results obtained on the 2 successive days (odd and even numbers) were in substantial agreement. They show that all 3 treatments greatly increased the proportion of water soluble reducing substances (presumably mainly sugars) present in the plants. A comparison of the results obtained before and after hydrolysis also shows little sucrose in any of the plants. It will also be noted that treatment with 1182 or HD 3 not only increased the concentration of reducing sugar but also the total amount of such sugar per plant. This is significant since the treated plants were only approximately half the size of the checks.

1182 has a formative effect on tomatoes and, by analogy with the action of related compounds (e.g., 2,4-dichlorophenoxyacetic acid) might be expected to influence the sugar metabolism. HD 3 and hydrogen peroxide, however, have no formative effect, nor are they likely to be translocated to any great extent. It therefore seems probable that their effect on sugar metabolism was a result of root injury. In this connection it should be noted that, at the concentration used, 1182 also caused considerable root injury.

It was considered likely that root injury might cause a reduction in the absorption of minerals by the plants. Spectrographic analyses were therefore made of individual plants taken from a third group on the twelfth day. These showed that the plants treated with 1182, HD 3 or hydrogen peroxide contained significantly less potassium, calcium, and phosphorus than the check plants ($P = .05$).

The experiments demonstrate that the treatment of tomato plants with any of the 3 compounds profoundly affects their metabolism. It is possible that these changes in metabolism may result in an increase in resistance.

INOCULATION OF TREATED TOMATO PLANTS THROUGH NEW UNTREATED ROOTS.—In the normal chemotherapy test the same roots which are inoculated have also been treated with compounds. If a reduction in wilt is subsequently noted, it is not possible to determine whether this occurred through inhibition of the pathogen in the roots or in other parts of the plant. To determine whether compounds applied to the roots could affect the fungus in other parts of the plant it was necessary to inoculate the plants in ways other than through the treated roots. Insertion of inoculum into stem wounds was tried but little infection resulted. Since inoculating roots of healthy plants gave consistently high infection, a method of growing plants with 2 root systems was studied. In this way 1 root system could be treated with the compounds and the other inoculated.

Adventitious roots were induced on the stems of the plants during treatment by surrounding parts of the stem in a 1:1 peat-loam mixture enclosed in a veneer wood box. The section enclosed was 1½ in. long and situated 1 in. above the base of the stem. The compounds under test were applied to the original roots in the main container only. The peat-loam mixture was frequently moistened with water but not supplied with nutrient. Adventitious roots up to 2 in. long were formed on the stem during the 2 wk. treatment period.

Ten plants were treated with HD 3, 10 with 1182 and 10 kept as checks. At the end of the treatment period the rooting boxes were removed from all the plants. Five plants from each group were inoculated through their original (treated) roots. The original roots and stem bases were removed from the other 5 plants which were then inoculated through the new

roots. Wilt records of these plants 26 days later are in Table 6.

TABLE 6.—*Leaf and vascular indices of plants inoculated through treated or new roots*

Treatment	Roots inoc.	Leaf grade	Vasc. grade
1182 (.0030%)	Adventitious	1.31	.36
	Original	.76	.13
HD 3 (.005%)	Adventitious	2.77	.84
	Original	.42	.07
Check	Adventitious	1.82	.54
	Original	3.51	.98

L.S.M.D. (P = .05) Leaf grade 1.00 Vascular grade 0.33

The wilt indices of individual plants were somewhat variable but certain definite conclusions can be drawn:

(a) Plants treated with HD 3 and then inoculated with *Fusarium* through adventitious roots showed no resistance. It must be concluded either that the HD 3 was ineffective in the stems above the new roots or that these new roots provided such a good substrate for the fungus that it was able to invade the stems very readily.

(b) The checks inoculated through the new roots became less severely diseased than those inoculated through the old roots.

(c) The growth of new roots on the stems of the treated plants did not impair the disease-reducing effect of either HD 3 or 1182 when the plants were inoculated in the normal manner (i.e., through the treated roots).

The results obtained by inoculating new roots on plants treated with 1182 are too variable to be conclusive. They suggest that this method of inoculation resulted in some increase in disease severity.

The method would appear of value in a study of the site of action of chemicals applied to the roots.

DISCUSSION.—It has been shown that if the roots of tomato plants are injured in any of 3 different ways the wilt symptoms induced by *Fusarium lycopersici* are greatly mitigated. One of these methods of root injury, viz., dipping the roots in hot water just before inoculation, acts by a single treatment which causes death of the inoculated roots.

The other 2 methods, viz., periodic root pruning and the application of root damaging compounds, however, cause root injury over a period before inoculation and do not usually kill the roots. Thus they bear a resemblance to the normal chemotherapeutant application. The varied nature of the treatments and the fact that they had only 1 obvious feature in common, viz, root injury, suggests that this caused the subsequent reduction in disease. The fact that the removal of most of the injured roots of plants treated with HD 3 and hydrogen peroxide did not give rise to an increase in disease suggests that the root injury had rendered the plants resistant. Evidence that these treatments also increased the carbohydrate content of aerial parts of the plants shows that the root-injury profoundly affected their metabolism. This change in metabolism may have been correlated with the increased resistance.

The application of root damaging chemicals to plants will presumably result in an unbalanced nutrient absorption, and this in turn may alter susceptibility of the host to disease. Thus tomato plants with a low nutrient supply are resistant to Fusarium wilt (14). A similar effect has also been demonstrated in the case of reduced nitrogen supply for Verticillium wilt of tomato (13) and of hops (12).

Evidently root injuring chemicals bring on changes in carbohydrate levels in plants. These will also affect susceptibility to disease, as has been shown in the case of Dutch elm disease (3,9) and cotton root rot (8).

One phase of the current research on chemotherapy involves the treatment of one part of a plant (usually the roots) with a compound and the assessment of its effect on disease occurring in another part. The object of this work is to discover systemic fungicides which can be translocated through the plant. The present investigation shows, however, that the chemical treatment of tomato roots may profoundly affect the resistance of other parts of the plant to attack by *Fusarium* and suggests that resistance to other diseases may be affected similarly. There is evidence that this resistance is not due to translocation of the applied compound in all cases, but that it may arise from an alteration in host metabolism.

Even without the gross effects of root damage reported herein, many compounds may be capable of changing the metabolism of the host plant and affecting its disease reaction. Thus, recent studies of the effect of maleic hydrazide, applied to tomatoes as a foliage spray without visible injury other than the normal stunting effect, have shown that this compound markedly increases the susceptibility of the inoculated plant to Fusarium wilt (15).

Even when the applied compound is translocated, as shown for griseofulvin (2), this possibility cannot be eliminated. It would thus seem advisable to exercise caution in interpreting the mechanism of disease reduction as a sole and direct effect of the applied compound on the pathogen.

The present work raises questions concerning what criteria should be used in defining a plant chemotherapeutant. A plant chemotherapeutant is sometimes regarded in the restricted sense of a systemically distributed compound which has a direct action upon a pathogen. Such a compound, although a chemotherapeutant, is defined more exactly as a systemic fungicide. Compounds which increase the resistance of a plant by altering its metabolism do not conform to this definition. Horsfall and Dimond (10, 11) have adopted a wider definition of a chemotherapeutant as a compound which, when applied to a plant, causes a reduction in disease, the action occurring within the

plant. In drawing up this definition they were primarily concerned with distinguishing between compounds which checked disease inside the plant and those which acted on the pathogen outside the plant. These latter are protectants. By this definition the important criterion is the location of the pathogen and not the location of the effective compound. The definition would, therefore, include those compounds which by purely physical effects change the metabolism of the plant even though they never penetrate the plant tissue.

The establishment of definitions in a relatively new field of research is always likely to prove difficult. It seems best to use a broad definition for convenience rather than to restrict one's research by a narrow definition. The present investigation provides an example of this.

The evidence cited in no way affects the possibility that certain chemicals can act as systemic fungicides. It does suggest, however, that in the case of Fusarium wilt of tomatoes many chemicals which reduce disease incidence do not act in this fashion. If the chemical treatment of plants will increase their resistance to disease this phenomenon would seem to be worthy of study as part of the broad field of plant chemotherapy. Certain aspects of this field will presumably become delimited as further information is obtained on the mechanism of action of various compounds. More restricted definitions of these other aspects will then become advisable.

SUMMARY

Certain types of injury to the roots of tomato plants before their inoculation with *F. lycopersici* resulted in greatly reduced disease-severity. Such injury was accomplished in 3 ways: (a) dipping the roots in hot water, (b) periodic root pruning, and (c) the application of sub-lethal doses of root-damaging compounds.

The various treatments had only 1 effect in common, viz., the production of root-injury. It therefore appears that disease reduction resulted from the root-injury *per se* and not from the specfific action of any individual treatment. Analyses of plants whose roots had been chemically injured showed that they had a higher soluble carbohydrate content and lower potassium calcium and phosphorus content than the uninjured check plants. Plants with chemically injured roots removed immediately before inoculation showed as little disease as those from which injured roots were not so removed. Checks with roots similarly removed became severely wilted. It is thus probable that the effect resulted from a change in host resistance and not merely from inhibition of the pathogen at the injured root surfaces. Some, but not all, of the chemicals selected by the tomato Fusarium wilt chemotherapy test cause considerable root injury (e.g., n-octadecyltrimethylammonium pentachlorophenate). Such injury must therefore be taken into account in considering the mode of action of these chemicals.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. ASSN. OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of analysis. 6th ed. 571-2. Washington, D. C. 1945.
2. BRAIN, P. W., J. M. WRIGHT, J. STUBBS, AND A. M. WAY. Uptake of antibiotics of soil microorganisms by plants. Nature **167**: 347-349. 1951.
3. CAROSELLI, N. E. AND A. W. FELDMAN. Dutch elm disease in young elm seedlings. Phytopath. **41**: 46-51. 1951.
4. CHAPMAN, R. A. Relation of specific chemotherapeutants to the infection court. (Abstr.) Phytopath. **41**: 4. 1951.
5. CHAPMAN, R. A., A. E. DIMOND, AND E. M. STODDARD. Assaying chemotherapeutants in the greenhouse. (Abstr.) Phytopath. **40**: 4. 1950.
6. DAVIS, D. Chemotherapeutic activity may be independent of fungitoxicity (Abstr.) Phytopath. **42**: 8. 1952.
7. DIMOND, A. E., D. DAVIS, R. A. CHAPMAN AND E. M. STODDARD. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agr. Exp. Sta. Bul. in press. 1952.
8. EATON, F. M. AND N. E. RIGLER. Influence of carbohydrate levels and root surface microfloras on Phymatotrichum root rot in cotton and maize plants. J. Agr. Res. **72**: 137-161. 1946.
9. FELDMAN, A. W. AND N. E. CAROSELLI. Soil and tree acidity in relation to susceptibility to Dutch elm disease (Abstr.) Phytopath. **41**: 12. 1951.
10. HORSFALL, J. G. AND A. E. DIMOND. Plant Chemotherapy. Trans. N. Y. Acad. Sci. **13**: 338-341. 1951.
11. HORSFALL, J. G. AND A. E. DIMOND. Plant Chemotherapy. Ann. Rev. Microbiology **5**: 209-222. 1951.
12. KEYWORTH, W. G. AND E. J. HEWITT. Verticillium wilt of the hop. V. The influence of nutrition on the reaction of the hop plant to infection with Verticillium albo-atrum. J. Hort. Sci. **24**: 219-227. 1948.
13. ROBERTS, F. M. Factors influencing infection of the tomato by Verticillium albo-atrum. Am. Appl. Biol. **30**: 327-331. 1943.
14. STODDARD, E. M. AND A. E. DIMOND. Influence of nutritional level on the susceptibility of tomatoes to fusarium wilt. Phytopath. **38**: 670-671. 1948.
15. WAGGONER, P. E. AND A. E. DIMOND. Effect of stunting agents, Fusarium lycopersici and maleic hydrazide, upon phosphorus distribution in tomato. (Abstr.) Phytopath. **42**: 24. 1952.

RELATION OF THE BLACK ROT PATHOGEN TO CABBAGE SEED [1]

A. A. Cook, R. H. Larson, and J. C. Walker

That the black rot pathogen (*Xanthomonas campestris* (Pam.) Dowson) may infest the seed of infected cabbage plants was first demonstrated by Harding, Stewart and Prucha (7) in New York state in 1904. Epidemics in Wisconsin in 1919 were found by Walker and Tisdale (14) to result directly from the use of contaminated seed. Monteith (9) suggested that the bacteria lodged on the seed coat during threshing and passed the winter in this location, but he did not disregard the possibility of seed infection. A hot-water seed treatment (50° C. for 25-33 min.) was found by Clayton (2) to control completely transmission of the disease in Brussels sprouts seed. This investigator also found the organism to remain viable for 3 yr. in naturally infected seed (4). Experiments by Richardson (10) with rutabaga seed revealed that the organism lost viability more rapidly on artificially contaminated seed than naturally diseased seed. In lots of the latter, however, the percentage of seeds with viable bacteria decreased with age of the seed.

The development of the disease in infected seed plants and the exact relation of the pathogen to the tissues have not been described. Therefore they were made the subject of this investigation. A preliminary report has been presented (6).

EXPERIMENTAL RESULTS.—*Disease development in cabbage seed plants.*—Experiments conducted by Harding, Stewart, and Prucha (7) showed that the black rot organism may pass the winter in cabbage plants saved for seed production. The pathogen was isolated by Smith (13) 30 cm. above the invasion point 15 mo. after inoculation from plants which had apparently recovered following the appearance of the first symptoms. The causal organism was found by Clayton (3) to overwinter in diseased cabbage and Brussels sprouts plants, but infected cauliflower plants never survived (1).

Over a period of many years several hundred headed cabbage plants have been selected for seed production annually in the field at Madison, Wisconsin, kept at 6° C. for about 3 mo., and planted in greenhouse pots in early January. Even though in the selection of such plants those showing symptoms of black rot were avoided, it was not uncommon to find that many showed signs of the disease after seed stems began to extend. Occasionally plants were killed before flowering but more often the first symptoms appeared on the lower cauline leaves after flowering had begun. Successively higher leaves usually succumbed until evidence of the disease appeared in the flowering branches. In these parts of the plant, darkening of veins became visible when the cortex of stem, pedicel, and pod were peeled back. Often pods were killed before seed matured. It was obvious that the organism, though present in the plant from the time of invasion at some time the previous season, had progressed slowly in the vascular system and that the plant had begun to bloom before leaf and stem symptoms were produced. When heading plants with external evidence of black rot were deliberately selected in the autumn, the same course of development followed, the disease signs usually not appearing in the seed plant until flower production was well under way. In over-wintered, infected, headed plants set out-of-doors at Madison the following spring, the disease followed the same course of development. Signs of black rot on seed plants are as a rule not distinct in comparison with those on plants in the vegetative stage of growth and therefore the disease can be overlooked readily in seed fields.

An experiment was set up to determine experimentally whether plants inoculated in the seedling stage might pass through the vegetative stage without outward signs of disease and show disease symptoms in the reproductive stage the second season. Four hundred seedlings were inoculated in the spring of 1950 in the greenhouse. This was done by sprinkling carborundum powder on 2 of the lowermost leaves and rubbing them with a glass spatula dipped frequently into a suspension of the pathogen. The plants were later transplanted out-of-doors and grown to the head stage. As soon as any sign of black rot appeared on plants they were discarded during the first season. In the autumn of 1950, about 300 plants which had remained free from external symptoms were pulled and after the outer leaves were stripped, they were stored at 6° C. On November 16 after 2 mo. storage they were set in pots in the greenhouse. The temperature of the greenhouse was kept at about 16° for the first 3 wk. then raised to 26°, which is about optimum for black rot development (6), for 4 wk. In order to encourage bolting and flower development the temperature was then kept at 16° to 20° for the next 3 mo., and finally it was again raised to about 26° for the period of final seed maturation.

Of about 300 plants which had remained symptomless during the previous summer 5 showed typical symptoms of disease after bolting. In one of these, typical external signs appeared in early January; and in another, in late January. A third plant showed symptoms in March and the remaining 2 did so in April. Many other plants were examined by cross-sectioning of stems as they approached maturity. Several showed slight internal vascular discoloration indicative of black rot but the pathogen was not isolated when the discolored tissue was plated out on agar.

In a previous paper (6) it was shown that in plants which become infected in an early vegetative stage external signs of the disease may disappear and plants may appear healthy for a considerable period, symptoms reappearing later in the season, often as margi-

[1] Accepted for publication March 1, 1952.

nal leaf lesions. The experiment just described showed that in occasional plants infected early, further external evidence of disease during the vegetative season may be absent while symptoms reappear in the late stages of flowering. The plants were kept for the major portion of the reproductive period at relatively low temperatures well below the optimum for disease development. Moreover disease symptoms became evident in the seed plants usually after a period of optimum temperature. In commercial seed production, plants are usually exposed to relatively cool temperatures during most of the reproductive period, the temperature not approaching the optimum for black rot symptom expression until after seed pods are developing. The importance of these facts concerning seed infection and seed production is obvious.

Relation of the pathogen to pods and seeds.—Material for this study was secured by inoculating partially developed pods through needle wounds in the pedicel. In such pods and those on naturally infected plants external evidence of infection appeared as shriveling and darkening of irregular areas (Fig. 1). Sections of the pod, each containing 1 seed still attached to the funiculus, were obtained by cutting transversely across the pod just back of the curved funiculus and across the apical tip of the seed. Usually the funiculus is irregularly curved in the direction away from the pedicel. The attachment of funiculi in a pod rather consistently alternates between both suture bundles and on both sides of the false membrane, which is often displaced by the developing seeds. The apical portion of each seed had always to be sacrificed to permit infiltration of solutions in preparation for sectioning (Fig. 2, A).

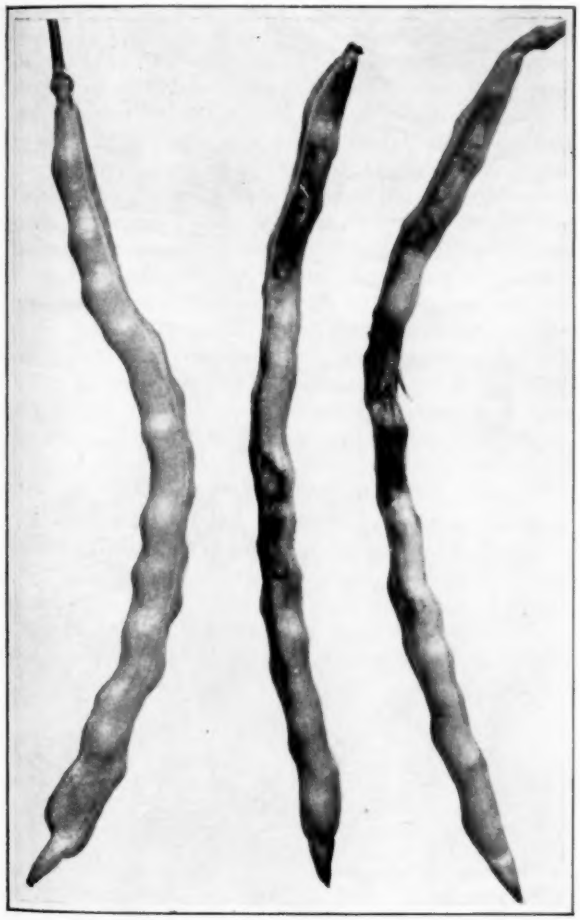


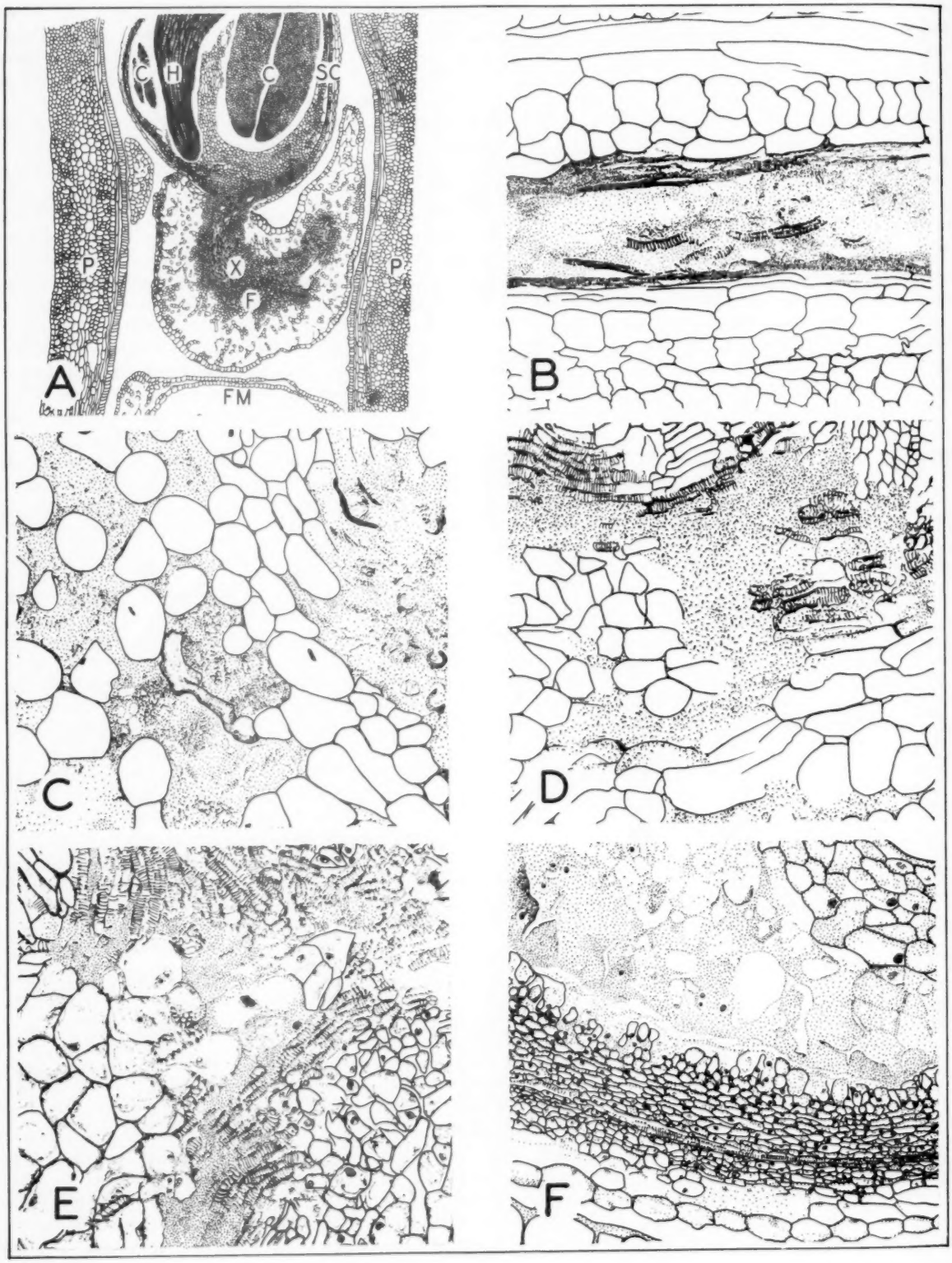
FIG. 1. Seed pods on the right from a naturally infected plant grown in the greenhouse. Healthy pod on left. ×1½.

All material was placed in formalin-aceto-alcohol immediately following collection and was evacuated with a suction flask for at least 10 min. Dehydration was by tertiary-butyl-alcohol method (12) and 56°–58° C. paraffin was used for embedding. The paraffin blocks were mounted so that sectioning was in a longitudinal direction with reference to both pod-suture veins and the funiculus. Sections were cut 11 microns in thickness and were affixed to the slide with Haupt's affixative (8). Staining was done in part by the Triarch quadruple technique (5) and in part by the safranin-fast-green method outlined by Riker and Riker (11).

Examination of the stained sections revealed that the bacteria frequently caused extensive lysigenous cavities in the xylem of the vascular bundles in the seed pod walls. In more severely affected areas the xylem was completely disrupted (Fig. 2, B). However, the activity of the pathogen was not usually found to be so destructive; the xylem generally was not appreciably disorganized. Intercellular invasion of the surrounding parenchymatous tissues was sometimes found but was never extensive. In several series of slides it was possible to follow the infection from a suture vein into the funiculus. Bacteria were observed in the vascular tissue at the junction of funiculus and suture vein as well as in many regions of the associated parenchymatous tissue (Fig. 2, C). Further invasion into the xylem of the funiculus was determined (Fig. 2, D), but in the more terminal portions of the funiculus relatively large lysigenous cavities were not often found. The xylem at the junction of funiculus and seed was frequently invaded (Fig. 2, E), but destruction by the pathogen was never observed to involve all of the vascular tissue. Although the immediately surrounding parenchymatous tissues were sometimes embraced, such invasion was apparently intercellular.

On close examination of that portion of the developing seed associated with the funiculus, some few xylem elements were seen to extend from the xylem of the funiculus into those tissues of the seed which become the seed coat at maturity (Fig. 2, F). Bacteria were never observed in the xylem of the seed coat, probably because there are but few elements present and these are always quite small in diameter. Even though the bacteria were not found to invade the xylem ultimately involved in the seed coat, the possibility of the pathogen remaining within the seed coat or in close association with it is not precluded. An arc

through the junction of funiculus and seed, formed by extending and joining the arcs prescribed by the outlines of both sides of the developing seed, transects a part of the xylem of the funiculus which was frequently found to be invaded. Thus, even though the major portion of the vascular elements of the funiculus



terminates abruptly in the region of attachment to the seed, some of the funicular xylem may be carried with the seed following threshing.

Recovery of the pathogen from infected seeds.—Seeds produced on naturally and artificially infected plants as well as artificially contaminated seeds were plated on nutrient-dextrose-agar. Approximately 575 well-filled seeds were obtained from naturally diseased plants and from pods of which the pedicels had been inoculated with *X. campestris*. The pathogen was recovered from only 2 seeds. When 100 aborted seeds from pods inoculated in the same manner were plated, the organism was recovered from 8. When healthy seeds dipped in a suspension of the organism were planted in quartz sand in 10-in. crocks, 95 of the 150 resulting plants showed symptoms of the disease. When 500 seeds taken at random from several naturally infected plants were treated in a similar manner, none gave evidence of being infected. However, when 50 seeds from one severely infected plant were planted, 3 of the plants were diseased.

Discussion.—In cabbage seed production vegetative plants in the heading stage are subjected to a cool period of 2 to 3 mo. either in situ in the field or in storage. After bolting, cool temperatures are most favorable for flowering and seed production. In practice, seed plants are seldom exposed to optimum temperature for black rot development until seed development is well under way. This study has shown that under this environment external black rot symptoms on seed plants are usually delayed until the flowering period, at which time more or less general systemic invasion of flowering stems, pedicels, and pods may have taken place. It is also significant that such symptoms may appear on plants which became infected early in the vegetative period but which did not develop external symptoms in the intervening period. While severely infected seed plants may be killed without flower production if exposed to favorable temperatures, they are of no importance in seed infection. It is the plant in which the disease appears just before seed maturation which is important in this regard.

The invasion of the young pods by the pathogen usually results in abortion of all seeds. In infected pods there are all gradations from this stage to plump germinable seeds. The organism advances from the suture vein into the xylem of the funiculus and presumably on occasion into the xylem of the seed coat, although this has not been demonstrated. It would appear, however, that in most cases infection is confined to the funiculus which commonly dries down and adheres to the seed. In such infected seeds the organism undoubtedly survives chemical treatments and yields only to hot-water treatment. There is also ample opportunity for surface contamination of seeds in the threshing process by organisms released from infected pods and from aborted seeds. These organisms are probably eradicated by chemical as well as by hot-water treatment.

Plating of seeds from infected pods shows that a surprisingly low percentage of such seeds are infected. This is interpreted as meaning that most seeds which are invaded abort and only rarely do germinable seeds contain the bacteria. These facts confirm what has often been observed by the writers in the field, namely that epidemics of black rot in cabbage may arise from a very few initially infected seedlings in a crowded seed bed when conditions for secondary spread are favorable.

It is evident that determination of the presence of such a minute, though dangerous, percentage of infected seeds in a given seed lot by plating bio-assay is very unreliable. Furthermore, requirement of a very highly efficient eradicative seed treatment, such as the hot-water method, for satisfactory control is obvious.

SUMMARY

Black rot symptoms develop most commonly on cabbage after the flowering stage is well advanced on seed plants infected during the vegetative period and grown under optimum environment for seed production. By this time systemic invasion of the vascular system of branches, pedicels, and pods is usually well advanced.

Invasion of the suture veins of the pods is followed by progress into the xylem of the funiculus, which commonly dries down and remains attached to the seed. Invasion of the seed coat through the funiculus is probable although it was not demonstrated histologically. In seeds with infected funiculi and seed coats the black rot organism probably resists chemical treatment and yields only to hot-water treatment. There is ample opportunity for surface contamination of seeds during the threshing process by organisms released from infected pod walls and from infected aborted

←

Fig. 2. A, Longitudinal section through a portion of a healthy cabbage seed pod illustrating the relation of the funiculus to the maturing seed and pod walls. C—cotyledons; F—funiculus; FM—false membrane; H—hypocotyl; P—pod; SC—seed coat; X-xylem in the funiculus. Appr. 94 ×. B, Vascular bundle in the wall of the seed pod in which the bacteria have completely disrupted all the xylem. The darker areas at the margins of the invaded region are an accummulation of dead bacteria, cell remains, etc. Appr. 185 ×. C, Mesophyll tissue at the base of the funiculus extensively invaded by the bacteria. Some remains of spiral thickenings can be seen in the upper right corner. Appr. 400 ×. D, Section through the bend of the funiculus in which the bacteria have caused considerable destruction of the xylem and have invaded the surrounding mesophyll tissue intercellularly. Appr. 400 ×. E, Section of the funiculus at its junction with the seed, showing the destruction incited by the bacteria in invaded xylem elements. Appr. 400 ×. F, Portion of an immature seed from a healthy pod, demonstrating the extension of some xylem elements into the immature seed coat from the funiculus. Appr. 185 ×.

seeds. Such organisms probably yield to chemical as well as to hot-water treatment.

Plating of seeds from infected pods showed that germinable seeds only rarely yielded viable black rot bacteria. This is interpreted as showing, in confirmation of field observations, that epidemics in the cabbage crop often arise from a very few initially infected seedlings in the seed bed under environment favorable for secondary spread. It is pointed out that bio-assay of seed lots for the black rot organism by the plating method is very unreliable. Furthermore a very highly efficient eradicative seed treatment is essential for satisfactory control.

DIVISION OF VEGETABLE CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
U. S. DEPARTMENT OF AGRICULTURE
AND
DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. CLAYTON, E. E. A progress report on black-rot investigations, with special reference to cauliflower on Long Island. (Abstr.) Phytopath. **14**: 24. 1924.
2. ———. Control of black-rot and black-leg of cruciferous crops by seed and seed bed treatments. (Abstr.) Phytopath. **14**: 24-25. 1924.
3. ———. Investigations of cauliflower diseases on Long Island. N. Y. State Agr. Exp. Sta. Bul. 506. 1924.
4. ———. Second progress report of black rot (Pseudomonas campestris) investigations on Long Island: seed infection and seasonal development. (Abstr.) Phytopath. **15**: 48-49. 1925.
5. CONANT, G. H. Triarch Botanical Products catalog, no. 8. p. 20. Ripon.
6. COOK, A. A., J. C. WALKER, AND R. H. LARSON. Studies on the disease cycle of black rot of crucifers. Phytopath. **42**: 162-167. 1952.
7. HARDING, H. A., F. C. STEWART, AND M. J. PRUCHA. Vitality of the cabbage black rot germ on cabbage seed. N. Y. State Agr. Exp. Sta. Bul. 251. 1904.
8. JOHANSEN, D. A. Plant microtechnique. New York. 1940.
9. MONTEITH, J., JR. Seed transmission and over wintering of cabbage black rot. (Abstr.) Phytopath. **11**: 53-54. 1921.
10. RICHARDSON, J. K. Black rot of rutabagas. Sci. Agr. **25**: 415-425. 1945.
11. RIKER, A. J., AND REGINA S. RIKER. Introduction to research on plant diseases. Chicago. 1936.
12. SASS, J. E. Elements of botanical microtechnique. New York. 1940.
13. SMITH, E. F. Bacteria in relation to plant diseases. Vol. II, pp. 300-334. Washington. 1911.
14. WALKER, J. C., AND W. B. TISDALE. Observations on seed transmission of the cabbage black rot organism. Phytopath. **10**: 175-177. 1920.

BACTERIAL CHALK ROT OF YELLOW AND PINK CALLAS[1]

Peter A. Ark, Mortimer P. Starr, and Donald D. Sutton

Yellow (*Zantedeschia Elliottiana*) and pink (*Z. Rhamanni*) callas are grown commercially for their corms and flowers in California, with the main producing area in Santa Cruz county. Annually quantities of the corms are destroyed or rendered unsaleable by a disease known as chalk rot. The disease is caused by a bacterium which leaves the starchy constituents of the corm undestroyed giving a chalky consistency to the corms after they have dried. This paper presents the results of the bacteriological, pathological and control studies of this disease.

Although Elliott (8) lists *Zantedeschia Elliottiana* as a host for *Erwinia aroideae*, a soft rot pathogen, the writers could find no mention of the original observation in references cited by Elliott. Ark and Tompkins (1) have described a soft rot of yellow calla leaves caused by *Erwinia carotovora*. A bacterial soft rot of white callas (*Z. aethiopica*) has been reported (2, 4, 3, 9, 15).

SYMPTOMS.—The disease appears in the field as a soft rot during the latter part of May and continues through the warm fall months. The flowers or leaves may show water-soaked streaks sometimes spreading laterally, eventually girdling the affected part and leading to general collapse. Sometimes the disease starts on the side of the growing point, but always moves toward it and eventually kills it, thus forming a cavity in the corm. Such corms show dark brown to black discoloration of the fibro-vascular bundles. Sometimes the presence of the disease in the field is evidenced by yellow-gray discoloration of the leaves which have a tendency to roll upward. Stunting of the plant with progressive yellowing and rolling of the leaves sometimes is present, especially in autumn. Such plants, when pulled gently, separate very easily from the corm and show a creamy yellow, slimy condition of the basal parts.

PATHOGENICITY—Pathogenicity tests were performed by (a) puncturing the leaves with a contaminated needle, (b) injecting a bacterial suspension into the corms, and (c) pouring a bacterial suspension into pots of pasteurized soil in which healthy corms were planted. The disease was successfully produced by the isolates from chalk rot by inoculation of yellow, pink and white callas. Severity of the infection depended on temperature and soil moisture. Maximal manifestation of rot symptoms was obtained at 27-32° C. with copious watering. Suppression of the disease was observed under conditions of low temperature and low soil moisture.

[1] Accepted for publication March 8, 1952.

EPIDEMIOLOGY—The divisions obtained from mother corms for planting often contain individuals in which the disease is present in a mild form. Planting by divisions affords one means of propagation of the disease in the field. Cormlets grown from seed become diseased when planted in contaminated soil. Large corms with late infections may appear sound at time of harvest but develop the disease when planted in warm greenhouses with heavy irrigation in order to force bulbs into flowering.

RESISTANCE AND HOST RANGE—Seeds of both yellow and pink callas collected from different areas in the state were grown in a greenhouse and tested by both needle and soil inoculations. Over a period of 3 years 25,000 seedlings of both yellow and pink callas and 6,000 large corms of both kinds were inoculated with virulent isolants of the organism; there was no indication of resistance to the chalk rot disease. The white calla (*Zantedeschia aethiopica*), as well as *Z. albomaculata*, *Z. melanoleuca*, and *Z. Pentlandi*, was found to be very susceptible. Infection and soft rot were produced also in carrot roots, celery (detached stalks), cucumber (both plant and fruit), egg plant, onion bulbs, parsley plants, potato tubers, squash (plant and fruit), tomato plants and fruits, turnip roots, and watermelons.

CONTROL.—Attempts at control consisted of chemical treatment of corms and cultural practices. Both yellow and pink calla corms were used. Chemicals used comprised mercuric chloride, 1:1000; lysol, 0.5 per cent; 8-quinolinol benzoate, 1:1000; sodium salt of *o*-hydroxydiphenyl, 1:1000; formalin, 2 per cent; and Roccal, 1:1000. Cultural methods consisted in fertilizing fields with cow manure at the rate of 8 tons to the acre; ammonium sulfate at 1 to 2 tons to the acre; 9-6-3 fertilizer at 2 tons to the acre and superphosphate at 2 tons to the acre. The treatments were made at the time of planting allowing an exposure of 20 min. to mercuric chloride and 60 min. to all other chemicals. Corms for the tests were taken from fields where the disease was severe. Results showed that very little benefit is derived from the chemical treatments. It was impossible to stop the disease once the corm was infected. A significant reduction in the amount of disease present in the field at the end of the growing season was shown by a 5 per cent incidence of infected corms in the plot fertilized with cow manure, as compared to 55 per cent in the untreated plot, 50 per cent in the ammonium sulfate-treated plot, 52 per cent in the plot dosed with 9-6-3, and 48 per cent in the superphosphate plot.

TAXONOMIC STUDIES OF THE PATHOGEN.—Twelve isolates and reisolates from typical chalk rot on yellow and pink callas were subjected to bacteriological determinative tests after rigorous checks to assure purity and virulence. Three cultures labeled *Erwinia carotovora*, isolated from potato rot, calla leaf (1), and pepper rot (11), and 2 cultures designated as *E. aroideae*, isolated from calla, and from tobacco stems, were studied at the same time. Although the description which follows is based upon the characteristics of the 12 cultures from calla chalk rot, 5 cultures from other sources are so close in their reactions as to be practically indistinguishable from the former. The methods used have been described elsewhere (6, 12).

Morphology. — Gram - negative, nonsporing, rod-shaped bacterium measuring 0.4-0.8 by 0.7-3.2 micra, and occurring singly or in pairs. It is actively motile with peritrichous arrangement of flagella.

Cultural characteristics.—Appearance of agar colonies and broth cultures is in agreement with the descriptions of *Erwinia carotovora* and *Erwinia aroideae* in Bergey's Manual (5).

Physiological reactions.—Gelatin stab: Early surface liquefaction, followed by infundibuliform liquefaction.

Pectate gel stab (12).—Saccate liquefaction.

Litmus milk—Acid reduction within 24 hours; curdling followed by separation of whey within 96 hours. Not peptonized within 6 weeks.

Indol is not produced.

Acetylmethylcarbinol is not produced.

Methyl red test is positive.

Grows in Koser's citrate medium; alkaline reaction is produced on Simmons' citrate agar.

Slight H_2S production can be detected with lead acetate paper over Thiotone broth (Baltimore Biological Laboratories) but not over beef peptone broth; H_2S production *cannot* be detected using lead acetate agar or Bacto triple sugar iron agar.

Not lipolytic by the spirit blue agar method (13).

Nitrite is produced from nitrate.

No diastatic activity can be detected on "soluble starch" agar by means of Lugol's iodine.

Grows in beef extract-peptone broth containing 8 per cent NaCl, but not 10 per cent NaCl.

Yellow pigment is produced in synthetic liquid medium containing tyrosine (12), but not the red-to-brown "tyrosinase reaction."

Grows in synthetic medium (14) containing ammonium chloride as the sole nitrogen source and various carbon sources with results as follows: All 12 isolates produce acid, (9 without gas, while 3 isolates consistently show slight gas production) from arabinose, xylose, rhamnose, galactose, glucose, mannose, cellobiose, lactose, melibiose[2], sucrose, trehalose, mannitol and salicin. Acid production without gas from fructose, maltose (very slow), raffinose, glycerol (slight), and inositol. Alkaline reaction from asparagine, sodium acetate, and sodium lactate. No growth, nor acid or gas, or very slight growth, in melezitose, dextrin, inulin, starch, methyl alcohol, ethyl alcohol, dulcitol, sorbitol, esculin, sodium butyrate, sodium tartrate, glycine, or urea.

The cultures from calla chalk rot thus fit the description (5, 7, 15) of either *Erwinia aroideae* or *E. carotovora* equally well.

The criteria used for separation of these 2 "species" is highly unsatisfactory. They have been separated on

[2] Gas production not determined.

the basis of gas production (10) but this distinction falls down in practice as in the present case where 3 calla cultures are gas-formers and 9 are non-aerogenic. Some so-called non-aerogenic cultures actually form large quantities of CO_2 (11). The two "species" have been "distinguished" on the basis of "characteristic" fermentations of various carbohydrates—a distinction which has varied from one writer to another, even in different reports of the same investigator. The inescapable conclusion is that differentiation between the 2 species is not supported by experimental evidence. Hence, we agree with Dowson (7) that *Erwinia aroideae* should be designated as a (usually "non-gas-forming") strain of *Erwinia carotovora*. Whether sufficiently high degree of host-specificity will be found to justify designation of the calla cultures as a *forma specialis* of *E. carotovora* is not now known—however, this seems unlikely on the basis of presently available evidence.

Discussion and conclusions.—The disease manifestations in the yellow and pink callas appear to be identical with those described for the white callas by Townsend (15). Soft rot of white callas is now very rare in California due probably to changed cultural practices. The similarity of the bacterial pathogens from all calla sources, and the fact that the writers' isolates from both yellow and pink calla corms produced the disease in the white calla corms, leaves little doubt as to the identity of the pathogens on all 3 types of calla.

Since the causal agent is a soil inhabitant, the possible control of the disease depends on microbiological relations existing in soils in which callas are grown. This is borne out by the fact that heavy manuring of chalk rot contaminated fields reduced the disease from 55 per cent to 5 per cent. It is quite probable that manuring of the soil stimulated and maintained the microflora inimical to the pathogen of the chalk rot. A survey of cultural practices among a few successful growers of callas confirms this premise.

SUMMARY

The organism causing chalk rot of yellow (*Zantedeschia Elliottiana*) and pink (*Z. Rhamanni*) calla corms is probably the same as the causal agent of soft rot of white callas (*Z. aethiopica*).

There appears no justifiable basis for naming the calla rot bacteria as a separate species; hence *Erwinia aroideae* should be considered as a (usually "non-gas-forming") strain of *Erwinia carotovora*.

The disease is most destructive on yellow and pink callas and constitutes a limiting factor in growing the callas for corms.

The disease is most severe under conditions of high temperature and high soil moisture.

Chemical treatments of the corms did not control the disease.

Heavy treatment of the soil with cow manure reduced the incidence of the disease considerably.

University of California College of Agriculture
Division of Plant Pathology, Berkeley, California
and
Division of Bacteriology, Davis, California

LITERATURE CITED

1. Ark, Peter A., and C. M. Tompkins. Soft rot of leaves and flowers of yellow calla (Zantedeschia Elliottiana) caused by Erwinia carotovora. U. S. Dept. Agr. Plant Dis. Rptr. **30**: 32. 1946.
2. Bewley, W. F. A bacterial disease of the Arum. Gard. Chron. 3(69): 152. 1921.
3. ———. Soft rot of the Arum. Cheshunt Exp. and Res. Sta. Ann. Rep. **7**: 39-40. 1922.
4. ———. Soft rot of the Arum. Cheshunt Exp. and Res. Sta. Ann. Rep. **10**: 74-75. 1925.
5. Breed, Robert S., E. G. D. Murray, and A. Parker Hitchens. Bergey's manual of determinative bacteriology. 6th ed. The Williams & Wilkins Co., Baltimore, Maryland. 1948.
6. Committee on Bacteriological Technic, Society of American Bacteriologists. Manual of methods for pure culture study of bacteria. Biotech Publications, Geneva, N. Y. 1923-1948.
7. Dowson, W. J. Manual of bacterial plant diseases. 183 pp. Ill. Adam and Charles Black, London. 1949.
8. Elliott, Charlotte. Manual of bacterial plant pathogens. 349 pp. The Williams and Wilkins Co., Baltimore, Maryland. 1930.
9. Halsted, B. D. Diseases of calla. New Jersey Agr. Exp. Sta. Rpt. for 1893: 399-401. 1894.
10. Harding, H. A., and W. J. Morse. The bacterial soft rots of certain vegetables. Part I. The mutual relationships of the causal organisms. Vermont Agr. Exp. Sta. Bul. **147**: 243-279. 1910.
11. Kraght, Albert J., and Mortimer P. Starr. Fermentation of galacturonic acid and glucose by a strain of Erwinia carotovora. Jour. Bact. In press.
12. Starr, Mortimer P. The causal agent of bacterial root and stem disease of guayule. Phytopath. **37**: 291-300. 1947.
13. ———, and Walter H. Burkholder. Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar and its taxonomic significance. Phytopath. **32**: 598-604. 1942.
14. ——— and Manley Mandel. The nutrition of phytopathogenic bacteria. IV. Minimal nutritive requirements of the genus Erwinia. Jour. Bact. **60**: 669-672. 1950.
15. Townsend, C. O. A soft rot of calla lily. U. S. Dept. Agr. B. P. I. Bul. **60**: 1-47. 1904.

DISTRIBUTION OF POTATO LATE BLIGHT AROUND INOCULUM SOURCES [1] [2]

Paul E. Waggoner [3]

An understanding of the manner of pathogen spread is a prerequisite for efficient forecasting and control of epiphytotics. Workers disagree as to the distribution of *Phytophthora infestans* sporangia around inoculum sources. For example, Limasset (6) and Bonde and Schultz (1) found that the number of blighted plants decreased to 0 within a few meters of the inoculum source. In opposition to this, Thomas (9) stated that sporangia of *P. infestans* capable of blighting potato leaves were transported many miles.

The study reported here proceeded as follows: first, distributions of blighted potato leaflets were observed around 3 isolated, known sources of sporangia. Second, these distributions were interpreted in terms of number of sporangia released by the sources, dilution of the clouds of sporangia by atmospheric turbulence, rates of deposition of air-borne sporangia, and ratio of sporangia deposited to percentage of leaflets diseased.

Observations of Disease Distribution.—Sources of *Phytophthora infestans* sporangia were produced near the centers of 3 plots of Cobbler potatoes growing on peat soil. Studies were begun when the plants were blooming. On June 19, an inoculum source consisting of 600 lesions was produced by inoculating a hill of potatoes at the center of the 1950 Clear Lake, Iowa plot. On July 7, an inoculum source consisting of 2 lesions was produced by inoculating a hill of potatoes near the center of the 1950 Wanatah, Indiana plot. After incubation periods of about 1 wk., extended periods of sporangia dispersal began from the 2 sources. The inoculum source of the third plot, 1949 Clear Lake, was of a different type. About 0.2 liter of a sporangial suspension was sprayed over a 360 by 180 centimeter area at the center of the plot just prior to a thundershower on June 20. The 1949 Clear Lake source was expected to spread sporangia over a shorter period of time and was expected to release a greater total number of spores than either of the other 2 sources. Results will be shown to confirm this.

Spatial distributions of living, fully-expanded leaflets with lesions were estimated by sampling at approximately weekly intervals. The 1950 Clear Lake plot consisted of 10 concentric circular rows 90 centimeters apart and the 1950 Wanatah and 1949 Clear Lake plots consisted of straight rows 90 centimeters apart. In each 22.5° arc of row in the 1950 Clear Lake or 90 centimeter length of row in the 1950 Wanatah and 1949 Clear Lake plot 2 stems were selected at random. The 10 uppermost and 10 lowermost living, fully-expanded leaflets on each stem were classified as diseased or not diseased depending upon the presence of macroscopic lesions. This method tends to give the time increment of disease because new leaves are expanded from the growing point and dead leaves are excluded from the sample.

1950 Clear Lake epiphytotic.—Five days after inoculation[4] 600 lesions appeared in the primary inoculum source. From day 5 until day 14 there occurred few hours of weather favorable[5] to late blight development. Sporangia dispersed from day 5 to 14 are assumed to have caused the macroscopic lesions observed on day 18. Figure 1 includes a map for the distribution of disease on day 18. No disease appeared in the tenth row and less than 2.5 per cent of the leaflets were diseased beyond the fourth row. Figure 2 shows that the disease gradient[6] on day 18 was steep. Only lower leaflets were diseased.

Frequencies of air movement directions at the nearby Mason City airport during the favorable hours from day 5 to 14 are shown in the lower portion of Figure 1. The arrows fly with the wind. This frequency diagram should represent conditions in the potato plot except for winds from the west through north which were obstructed by a road embankment to the west and a windbreak to the north of the plot. Considering the obstructions to air movement in the plot, it is evident from Figure 1 that the directions of greatest disease extension are in the directions of most frequent unobstructed air movement.

By day 24, leaves previously diseased had died and only 1 new lesion was found in the plot. Because only a few hours of favorable weather occurred between days 11 and 20, few sporangia had been dispersed. Leaves diseased on day 18 had died and were excluded from the sample; only a single new lesion appeared. Hence, there was a decrease rather than an increase with time in the number of diseased leaflets.

During the weeks following day 24 the weather was more favorable for blight, which increased in the plot despite near extinction of the disease on day 24. Figure 1 shows that on day 42 more than 2.5 per cent of the leaflets were diseased in most of the plot. The area with the most diseased leaflets was toward the north and the windbreak. An east to west strip of

[1] Accepted for publication March 7, 1952.

Thanks are due to Dr. J. R. Wallin and Dr. R. W. Samson for aid in the field work. The aid of Prof. O. Kempthorne, Mr. J. E. McDonald, and the staff of The Conn. Agr. Exp. Station was invaluable in the interpretation of the observations. The partial financial support of the Division of Mycology and Disease Survey, U. S. Dept. of Agriculture is acknowledged.

[2] Published with the approval of the Director, the Connecticut Agricultural Experiment Station.

[3] Assistant Plant Pathologist, The Connecticut Agricultural Experiment Station, formerly Agent, Division of Mycology and Disease Survey, U. S. Department of Agriculture.

[4] Time is counted from date of inoculation.

[5] Hours with relative humidity equal to or greater than 90 per cent and temperature equal to or less than 70° F. were assumed to be favorable.

[6] Disease gradient is defined here as the decrease with distance from the primary source in percentage of leaflets diseased.

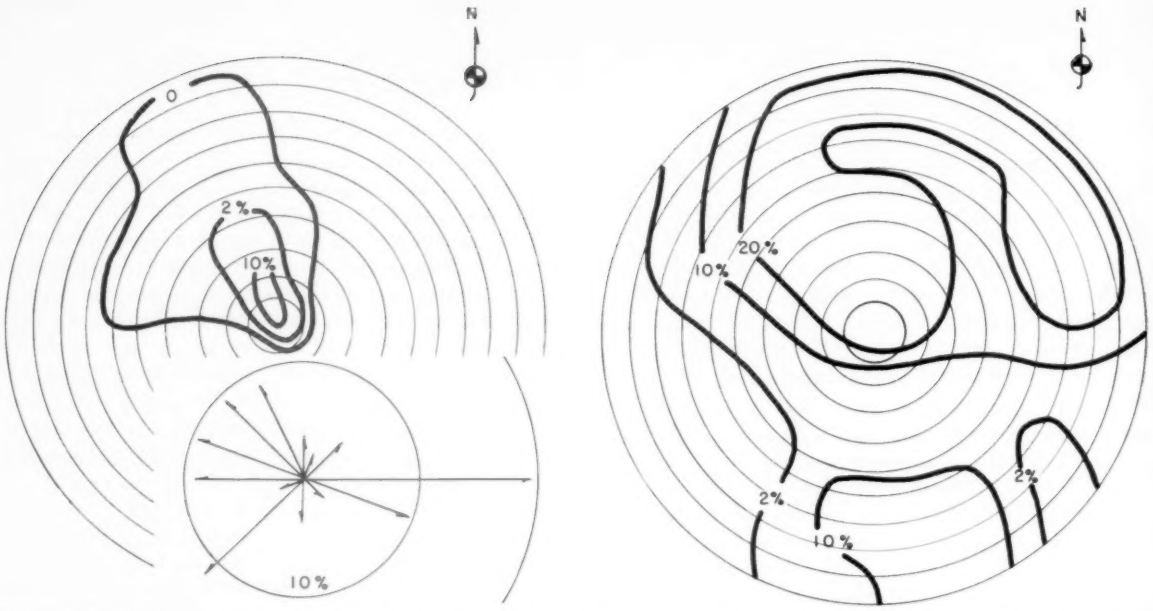


FIG. 1. Left: Upper portion: Map of the 10 concentric circular rows of the 1950 Clear Lake plot showing the percentage of leaflets diseased on day 18. Lower portion: Frequencies of wind directions at the Mason City airport during favorable hours from day 5 to 14. Arrows fly with the wind. Right: Map of the 1950 Clear Lake plot showing the percentage of leaflets diseased on day 42.

sparse planting between 3 and 5 meters south of the primary source was caused by poor drainage. This strip appears in Figure 1 (right) as an area of few diseased leaflets.

The disease gradient around the primary source on day 42 is shown in the right half of Figure 2. Comparison of the 2 halves of the figure shows that secondary spread flattened the disease gradient. On day 42 about the same numbers of upper and lower leaflets were diseased.

1949 Clear Lake epiphytotic.—After the release of sporangial suspension at the center of the 1949 plot, a thundershower occurred. The leaves did not dry until the next morning. On day 9 late blight was observed near the source. A windbreak north of the plot caused the maximum number of diseased leaflets to appear north of the source. The steep disease gradient on day 9 is shown in Figure 3. Distances were measured from the edge of the sprayed area.

The number of diseased leaflets in the 1949 plot increased steadily with time and the disease gradient became flatter. By day 38 the gradient became 0. About the same numbers of upper and lower leaves were diseased on that date.

1950 Wanatah epiphytotic.—In the Wanatah plot more hours of favorable weather occurred before day 15 than had occurred during a like period in the other plots. Therefore, the number of viable sporangia produced per lesion in the primary source was greater in the 1950 Wanatah source than in the 1950 Clear Lake source. Consequently, the ratio of the number of diseased leaflets observed in the Wanatah plot on day

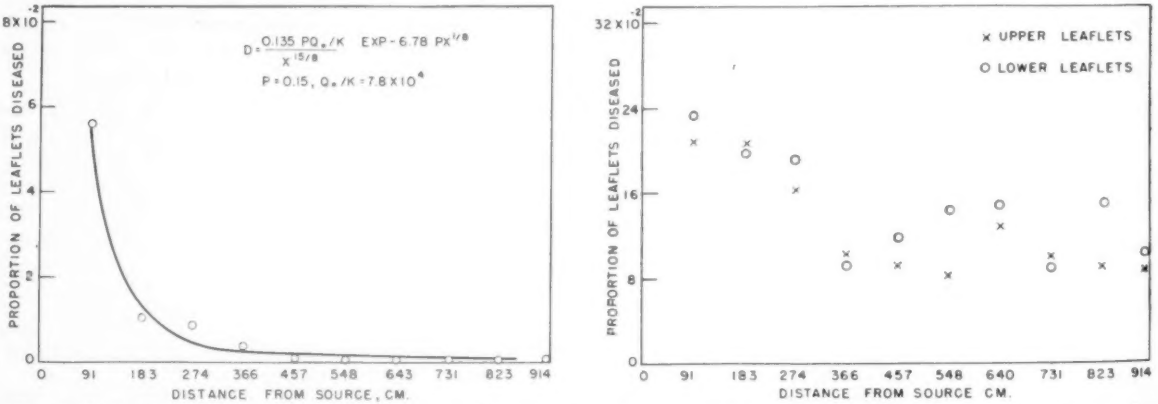

8X10⁻²
PROPORTION OF LEAFLETS DISEASED
D = 0.135 PQ₀/K EXP - 6.78 PX^1/8 / X^15/8
P = 0.15, Q₀/K = 7.8X10⁴
0 91 183 274 366 457 548 643 731 823 914
DISTANCE FROM SOURCE, CM.
32X10⁻²
PROPORTION OF LEAFLETS DISEASED
× UPPER LEAFLETS
O LOWER LEAFLETS
0 91 183 274 366 457 548 640 731 823 914
DISTANCE FROM SOURCE CM.

FIG. 2. Left: Proportion of leaflets diseased at distances from the primary source of the 1950 Clear Lake plot on day 18. Right: Proportion of leaflets diseased at distances from the primary source of the 1950 Clear Lake plot on day 42.

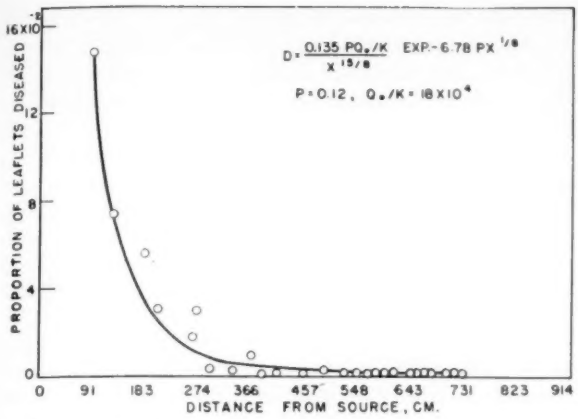


FIG. 3. Proportion of leaflets diseased at distances from the primary source of the 1949 Clear Lake plot on day 9.

19 to the number observed in the 1950 Clear Lake plot on day 18 was much higher than would be expected from the simple ratio of the number of lesions in the 2 sources. No lesions were observed beyond 204 centimeters from the Wanatah source. More lower than upper leaflets were infected.

Favorable weather continued. By day 34 late blight was present in all the sampling units of the 13 by 13 meter plot. Late blight was also present to the furthest boundary of the adjacent plots, a distance of 48 meters from the primary source. About equal numbers of upper and lower leaflets were diseased.

CHARACTERISTICS OF SPORE DISPERSAL INFERRED FROM DISEASE DISTRIBUTIONS.—Because the distribution of diseased leaflets reflects the distribution of pathogenic spores, characteristics of sporangia dispersal can be inferred from the observations given. Interpretation is begun by proposing a mathematical model for percentage of leaflets diseased, in which percentage of leaflets diseased is a function of parameters that depend upon characteristics of the sporangia dispersal process. Least squares estimates of the parameters are obtained from the disease observations of the preceding section. This analysis provides more precise information about the sporangia dispersal process than is possible by qualitative interpretation of disease in terms of sporangia deposition.

Mathematical model for percentage of leaflets diseased.—A mathematical model for the deposition of air-borne spores has been derived by Gregory (5). The model derived here differs from Gregory's because it takes into account the damping of vertical turbulence by the earth's surface and makes explicit the relation between spore deposition and infection.

A single puff of spores emitted from an instantaneous point source comprises a cloud that expands as the cloud moves. Assuming that the movement of the eddies bearing the spores is random, the concentration of spores is expected to be normally distributed in each of the 3 dimensions x, y, and z measured downwind, crosswind, and vertically from the center of the moving cloud. The length, width, and height of the expanding cloud are described by the variances of the spore concentration distributions in the 3 dimensions.

The variance V of the distribution of spore concentrations crosswind must be a function of X, the distance the center of the cloud has moved downwind. Sutton (8) proposed that

$$V = 1/2\, C^2 X^m$$

where C is a constant and m is a function of turbulence. From an analysis[7] of Stepanov's data Gregory (5) found m equal to 1.76 and C equal to 1.2 cm.$^{1/8}$. Wilson and Baker's (11) data lead to values between 1.5 and 2 for m and between 0.6 and 1.0 cm.$^{1/8}$ for C. Values of m and C were here set equal to 1.75 and 1 cm.$^{1/8}$.

Downwind and vertical variances are known to be proportional to the crosswind variance. Wilson and Baker (11) studying spore dispersal and Scrase (7) studying air movement found that the variances of vertical distribution of spore concentration and air movement were 4/9 of the horizontal variances. Scrase (7) found that the downwind and crosswind variances were equal. Therefore, in the model the variance of the concentration distribution in the vertical direction was set equal to 4/9 of variances in the horizontal directions.

Correlations are known to exist between deviations in vertical and horizontal air movement. Scrase (7) found a negative correlation between vertical and downwind variations but no correlation between cross and downwind variations. For the sake of simplicity in the present study it was necessary to assume that deviations in the 3 directions were uncorrelated.

The foregoing assumptions specify a trivariate normal distribution for spore concentration, S, and fix the means, variances, and covariances. The specified distribution is

$$S = \frac{3Q}{2\pi^{1/2} X^{3m/2}}\ e^{-X^{-m}(r^2 + 9z^2/4)}$$

Where $r^2 = x^2 + y^2$ and Q is the number of spores still in the cloud when its center has travelled a distance X.

Sporangia are deposited from the cloud as it moves away from the source. Hence, the number of spores in the cloud decreases from Q_0 at the source to Q after the cloud has traveled a distance X. The rate at which Q decreases is a function of the deposition rate. The deposition rate is the ratio of the number of spores deposited per cm^2. of the horizontal plane to the number of spores passing over that cm^2. Using the above distribution of S and introducing the source strength and the deposition rate, an expression can

[7] Gregory's estimate of C equal to 0.6 is in units of m.$^{1/8}$. The 0.6 m.$^{1/8}$ equals 1.2 cm.$^{1/8}$.

be derived for the mean number of spores deposited on a cm^2. of horizontal surface at distance X from an instantaneous point source. This derivation follows Gregory's (5) reasoning. By assuming that deposition from a continuous point source is equal to the sum of the depositions from a large number of instantaneous sources, the above model for an instantaneous source becomes applicable to spore deposition from the continuous sources encountered in the field studies.

The proportion of diseased leaflets is expected to be linearly proportional to the number of spores deposited when substrate and environment are uniform over the area studied and when spore concentration is low. These conditions were reasonably satisfied by the first observations in each of the 3 plots. Using the expression for spore deposition described in the previous paragraph and introducing the proportionality between spore deposition and proportion of leaflets diseased, an expression for proportion of leaflets diseased, D, can be written as follows:

$$D = \frac{0.135p(Q_0/k)}{X^{15/8}} e^{-6.78pX^{1/8}}$$

where k is the ratio of spores deposited per cm^2. to the proportion of leaflets diseased, Q_0 is the number of spores released at the source, and p is the deposition rate. The parameters p and Q_0/k characterize the dispersal of spores and consequent disease production. The parameters characterizing spore dispersal can be estimated from observations of D and X, i.e., disease distribution.

Characteristics of sporangia dispersal in the 3 plots.—The parameters p and Q_0/k were estimated from observations of disease distribution resulting from dispersal of sporangia from sources that were approximately points. The observations in the 1950 Clear Lake plot on day 18 and in the 1949 Clear Lake plot on day 9 were suitable. The parameters were estimated by the method of least squares from untransformed, mean percentages of leaflets diseased at 10 distances from the 1950 and 26 distances from the 1949 source. The estimates and corresponding curves appear in Figures 2 and 3. The mean squares of deviations from the curves are 4×10^{-6} and 46×10^{-6} for the 1950 and 1949 Clear Lake data, respectively. The longer period of dispersal from the 1950 Clear Lake source led to a disease distribution that more nearly approached a smooth curve than did the shorter period of dispersal from the 1949 source.

Diseased leaflets were observed at too few distances from the Wanatah source on day 19 to provide a worthwhile estimate of p, the deposition rate, because the estimate of this parameter depends upon the curvature of the relation between disease and distance. However, by setting p for the Wanatah plot equal to the estimate of p for the 1949 plot, 0.12, Q_0/k for the Wanatah plot was estimated as 3×10^4.

Before the estimates of p can be interpreted, some understanding must be gained of the factors affecting this rate of deposition. Deposition is expected to increase when horizontal air movement slows and the spores are over a given cm^2. of surface for a longer period. Therefore, p must be inversely proportional to horizontal wind speed. This proportionality is recognized in Sutton's (8) and Winsche's (12) models for dispersal of material from continuous sources. Deposition is expected to increase when downward spore movements increase. Therefore, p must be proportional to downward spore velocity. Finally, although the model has been derived for deposition on unit area of a uniform, plane surface surrounding the source, deposition in the field occurs throughout a volume that extends from the soil surface, to plant height and has unit horizontal cross-section area. If the spores not caught by leaves remain air-borne, p must be proportional to the spore catching efficiency of the leaves. The above assumptions concerning the nature of the deposition rate can be summarized by setting p proportional to v/u and to E where v is downward spore velocity, u is horizontal wind speed, and E is the spore catching efficiency of the leaves.

If the above assumption concerning the nature of p is true, the near equality of the estimates of p for the 1949 and 1950 Clear Lake plots is interesting. The value of E in 1950 was expected to be greater than in 1949. This was expected because the 1950 sporangia were released near the base of the plants and would be filtered out rapidly by adjacent plants while the 1949 sporangia were released near the tops of the plants. The near equality of p and the inequality of E in the 2 plots led to the conclusion that v/u was larger in 1949 than in 1950. The v/u could have been increased in 1949 by turbulence due to the thundershower or by increased settling velocities of the sporangia due to their suspension in water droplets. Further studies of the parameter p are required to indicate the disease danger from spores in the air. In other words, airborne spores will not produce disease until deposited on a suscept. Therefore, attempts should be made to understand the deposition rate.

Estimates of Q_0/k for the 3 plots were in the same order as the expected source strengths. However, the ratios of the Q_0/k were not equal to the expected ratios of the source strengths. This was partially due to variations in k, the ratio of sporangia deposited to proportion of leaflets diseased. The reciprocal, 1/k, is the disease-producing efficiency of the sporangia. For example, the Q_0/k for the Wanatah plot was fully ½ the value for the 1950 Clear Lake plot despite the presence of only 2/600 as many lesions in the Wanatah as in the Clear Lake source. This result was attributed to the more favorable weather in the Wanatah plot, causing both the production of more sporangia per lesion in the source and the higher disease-producing efficiency of the sporangia.

The 1949 Clear Lake source, 0.2 liters of sporangial suspension sprayed near the tops of the plants, led to

a higher $Q/_0k$ than the 600 lesions of the 1950 Clear Lake source. Three factors contributed to this effect. First, the viability of sporangia was maintained in 1949 by their suspension in water. Second, all available sporangia were dispersed in 1949 while in 1950 many no doubt remained on the source leaves. Third, many sporangia that were dispersed from the lesions of the 1950 source, which was near the ground, probably settled out on the ground. Two factors tended to counteract the above 3. First, sporangia from the low position of the 1950 source were more apt to settle on the underside of the leaves which, according to Crosier (2), is the more susceptible side. Second, sporangia from the low position of the 1950 source were more apt to settle on the lower leaves where most primary infection occurred. Evidently the 3 factors leading to greater source strength and disease-producing efficiency of sporangia in 1949 were more effective than the 2 counteracting factors. Consequently, spores introduced into the turbulent layer of air near the tops of the plants at the beginning of a period of favorable weather gave rise to more infected leaves than spores from a large number of lesions present for an extended period of time in the layer of air near the ground.

Studies on the behavior of the parameter Q_0/k are required to indicate the disease danger from different types of pathogen sources and the environments in which the sources operate. For example, studies begun by Dimock (3, 4) on the effect of pest control sprays should be continued. A spray blast probably causes the release of more spores, i.e., increases Q_0. Wetting of spores and leaves by the spray probably increases disease producing efficiency, 1/k, of the spores. If these 2 factors occur to a greater extent than the decrease of 1/k by fungitoxicity of the spray, Q_0/k and disease in a field would be increased by spray application. Evidently, dispersal of spores and wetting of spores and leaves by the spray as well as fungitoxicity of the spray must be studied in evaluating a spray control program. The mathematical hypothesis provides an efficient means of designing and analyzing these studies.

From the mathematical hypothesis, deductions can be made that bear upon the disagreement concerning distribution of *P. infestans* sporangia. The deductions are confidently accepted because the hypothesis agrees with the observations given. The hypothesis predicts that the probability is about 1 in 1000 of a leaflet becoming diseased at 10 meters from a source similar to the one of 1949. At 1 mile the probability of a leaflet becoming diseased would be less than 1 in 10 million. The probability would be increased when the source released more spores, when the host was downwind from the source, and when many leaflets and long exposures were concerned. The increased probability of infection associated with wet weather is illustrated by the rapid secondary spread in the Wanatah plot. On the other hand, the probability would be sharply reduced if the atmosphere were not saturated (2). Therefore, catching a pathogenic sporangium on a single leaflet—or microscope slide—at a distance of 1 mile from a source of sporangia would be an extremely rare event. The probability of a single pathogenic sporangium being deposited in a 1 acre field located 1 mile from a source similar to the one in 1949 would be of the order of $\frac{1}{3}$. This probability must be greatly reduced by desiccation of the air-borne spores.

Several practical conclusions can be derived from the studies reported here. Decrease in number of diseased plants is rapid as one goes away from a source of inoculum that has resulted in primary infection only. Hence, only small amounts of infection will appear on plants not in the vicinity of a source of inoculum. Because proximity of a source is relatively more important than strength of the source, spatial distribution of diseased plants becomes more uniform as secondary infection progresses.

Early infection is largely limited to lower leaves. As secondary infection proceeds, more upper leaves are involved. Waggoner and Shaw (10) discuss the microclimatic reasons for this phenomenon.

Because spore dispersal is influenced by air movement, late blight will develop most rapidly on the side of the source toward which the wind blows during favorable periods. In a sheltered field this side will be toward the shelter.

SUMMARY

Spatial distribution of diseased leaflets around 3 isolated sources of *Phytophthora infestans* was observed at approximately weekly intervals.

Dispersal of the fungus as traced by late blight development was greatest in the direction of air movement. Primary infection from a source was limited to an area within 10 meters of the source. Secondary infection flattened the disease gradient. Early infection was largely confined to lower leaves; later infection involved both upper and lower leaves. Although a period of unfavorable weather caused the near extinction of the fungus in a plot, the fungus spread widely during a later period of favorable weather.

A modification of Gregory's (5) hypothesis for the deposition of air-borne spores permitted a precise interpretation of the observations of primary infection in terms of sporangia dispersal. The hypothesis is applicable only to primary infection because it is derived for the case of a point source of spores. When sporangia were dispersed over an extended period of time, the disease distribution conformed more closely to the hypothesis. Rate of deposition of sporangia was evidently increased by increasing their downward velocity by vertical air movement or by suspending them in water droplets. Infection produced by a source of inoculum increased when number of lesions in the source increased. Infection evidently was increased in 2 ways by weather favorable to late blight. First, the number of sporangia produced per lesion in the

source was increased. Second, the disease-producing efficiency of the sporangia was increased.

Probability of a leaflet becoming diseased at distances from sources similar to the ones studied can be estimated from the hypothesis. Deposition of a pathogenic sporangium on a leaflet or slide at 1 mile from a source is deduced to be a rare event. Deposition of a pathogenic sporangium in a 1 acre field 1 mile from a source is deduced to be common. The probability of disease being caused by sporangia deposited at a considerable distance from the source must be sharply reduced by a non-saturated atmosphere.

CONNECTICUT AGRICULTURE EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. BONDE, R. AND E. S. SCHULTZ. Potato refuse piles as a factor in the dissemination of late blight. Maine Agr. Exp. Sta. Bul. 205. 1943.
2. CROSIER, W. Studies in the biology of Phytophthora infestans. N. Y. (Cornell) Agr. Exp. Sta., Mem. 155. 1934.
3. DIMOCK, A. W. The dispersal of viable fungus spores by insecticides. Phytopath. **41**: 152-156. 1951.
4. ————. The dispersal of viable spores of phytopathogenic fungi by fungicidal sprays. Phytopath. **41**: 157-163. 1951.
5. GREGORY, P. H. The dispersion of air-borne spores. Trans. Brit. Myc. Soc. **28**: 26-72. 1945.
6. LIMASSET, P. Recherches sur le P. infestans (Mont.) de B. Ann. Epiphyt. **5**: 21-39. 1939.
7. SCRASE, F. J. Some characteristics of eddy motion in the atmosphere. Gt. Brit. Met. Off. Geophys. Mem. **52**: 3-16. 1930.
8. SUTTON, O. G. A theory of eddy diffusion in the atmosphere. Proc. Roy. Soc. A. **135**: 143-165. 1932.
9. THOMAS, W. D. Factors influencing the epidemiology of late blight of potatoes. Unpubl. PhD. Thesis. Minneapolis, Minn., Univ. of Minn. Library. 1947.
10. WAGGONER, PAUL E. AND R. H. SHAW. Temperature of potato and tomato leaves. Plant Phys. In press.
11. WILSON, E. E. AND G. A. BAKER. Some aspects of the aerial dissemination of spores with special reference to conidia of Sclerotinia laxa. Jour. Agr. Res. [U.S.] **72**: 302-327. 1946.
12. WINSCHE, W. E. Deposition of non-volatile aerosol clouds in open and forested areas. O.S.R.D. Informal Rept. **10**: 4-55. 1944.

COWPEA AS AN ASSAY HOST FOR CUCUMBER VIRUS 1 [1]

W. H. Sill, Jr. and J. C. Walker

Among the plants which give a local-lesion response when inoculated with certain strains of cucumber virus 1 are watermelon (*Citrullus vulgaris* L.) (cotyledons only) (12), *Amaranthus caudatus* L. (4), cowpea (*Vigna sinensis* Savi.) (7), *Chenopodium hybridum* L. (8) and *Lupinus polyphyllus* Lindl. (3). Of these the cowpea seemed to be most promising for quantitative work, but the local-lesion reaction of individual plants to a given strain of the virus was usually very erratic. Before dependable quantitative use of the plant could be made in an investigation of virus concentration in resistant and susceptible cucumber plants (*Cucumis sativus* L.) a study of various factors affecting lesion development was undertaken, the results of which are presented in this paper.

EXPERIMENTAL RESULTS.—The carborundum-rubbing method was used since it increased consistently the lesion numbers. Leaves were supported for all inoculations upon the surface of folded paper towels, and were rinsed with water immediately after inoculation. The glass spatula and the muslin swab were compared as inoculation instruments. Since there was little or no difference between the 2, the glass spatula was used in subsequent inoculations. After a comparison of 1, 2 and 3 strokes and a circular motion of the spatula, the last method was chosen because it gave high lesion numbers, little plant injury, and could be applied most rapidly and uniformly. The amount of inoculum used at a given dilution had little, if any, effect upon lesion numbers. A dilution of 1 to 10 with 0.1 M phosphate buffer at pH 7.0 was chosen as the standard inoculum because in many trials it yielded greater lesion numbers than higher or lower dilutions. Although the half-leaf method was used successfully, it was found to have important disadvantages in the relatively small size of leaves and in the smooth leaf surface which resulted in a tendency of the inoculum to flow across the midrib. Opposite primary leaves, compared in 5 different uniformity trials, were found to give nearly equal average numbers of lesions. There was no statistical significance in the difference in the number of lesions between opposite leaves in any trial.

The local-lesion response of 12 varieties of cowpea was tested. Varieties Black, California Blackeye, and Brown Crowder were most satisfactory of those tested; from these the variety Black was finally chosen because it gave most local lesions under favorable conditions.

Several strains of the virus were compared. These included Price's No. 6 strain (7); southern celery mosaic as described by Wellman (11); a strain isolated from spinach, designated herein as Y and used earlier by Kuntz and Walker (4); a strain isolated from the ornamental crucifer, dame's violet, by Pound and Walker (6) and designated herein as X; and Doolittle's type strain. Each strain was inoculated to uniform, young plants of *Nicotiana tabacum*, var. Havana 38. The assay inoculum was then obtained from these source plants. Plants of uniform age and identical inoculation dates were used for each comparative as-

[1] Accepted for publication March 17, 1952.
This investigation was supported in part by a grant from the University Research Committee.

say. Strains differed widely in the average number of lesions per leaf (Table 1). Strain Y was chosen for further work because of the high number of lesions produced.

TABLE 1.—*Comparison of number of local lesions on cowpea caused by 6 strains of cucumber virus 1*

Trial No.	No. of plants inoculated	Ave. number of lesions per leaf for strain indicated				
		Price's No. 6	Southern celery	Y	X	Doolittle's strain
1	16	10	3	46	4	52
2	33	28	12	70	3	61
3	25	7	3	56	2	62
4	36	11	2	47	8	21
5	45	46		31	5	26

Choice of virus source plant.—The choice of a virus source plant for a standard or control inoculum becomes very important in quantitative work since it has been shown repeatedly that the concentration of virus may differ widely from host to host. Infected cucumber was assayed first. Inoculum was prepared by macerating all the leaves of the plant, filtering the extract through 2 layers of cheesecloth, and diluting it as previously described. Lesions were formed only rarely even when the extract was obtained from severely infected plants. This was owing to an inhibitive substance in cucumber which will be described in a later paper. Infected plants of *Nicotiana tabacum* L., *N. glutinosa* L., and the F_1 hybrid of *N. tabacum* × *N. glutinosa* were compared next. Lesion averages were 82 for *N. tabacum* (5 trials), 46 for *N. glutinosa* (1 trial), and 11 for the hybrid (4 trials). *N. tabacum* was chosen as the most satisfactory source plant for the virus for further studies. Uniform 3- to 4-leaf tobacco plants growing at 20° to 25° C. grown in an unshaded greenhouse with natural sunlight augmented by fluorescent lamps during winter months were inoculated about 1 wk. after being transplanted. Inoculum was secured from such plants within 1 wk. after the appearance of conspicuous symptoms.

The method described above was compared with one which consisted of obtaining tissue samples with a cork borer from chlorotic areas of infected tobacco plants. These samples were macerated with 10 parts (by weight) of buffer solution. Assays on opposite cowpea leaves in 7 trials yielded an average of 121 lesions with the standard method and 129 with the cork-borer method. Following the method described by McKinney (5) several samples of tobacco source plants were desiccated, ground, mixed, and assayed at various times over a period of 1 year. No more consistent lesion counts were obtained from the desiccated inoculum source than from the standard inoculum taken from living plants.

Age and nutrition of test plant.—Primary leaves of plants of varying ages and also trifoliate leaves of older plants were inoculated with aliquots of the same inoculum. As shown by Table 2, primary leaves of young plants, inoculated prior to the expansion of the first trifoliate leaf, were very susceptible as compared to old unifoliate leaves and trifoliate leaves. Lesions were found on the latter only rarely even when extracts of high virus titer were used. Several trials were made to determine the effect of nutrition of the cowpea plant upon local-lesion response. Plants were grown in washed quartz sand, watered with a range of concentrations of balanced Hoagland solution and with solutions in which nitrogen, phosphorus, and potassium were at high and low levels. No significant differences in the number of local lesions per plant were found between any of the solutions used.

TABLE 2. —*Effect of age of cowpea plant upon number of local lesions produced when inoculated with cucumber virus 1*

Trial No.	No. of plants inoculated	Average lesions per leaf		
		Unifoliate leaves		
		12 to 15 day old plants	15 to 25 day old plants	Trifoliate leaves of older plants
1	11	34	8	0
2	18	58	3	0
3	8	102	2	0
4	7	110	12	–
5	7	72	13	–
6	5	177	21	–
7	18	12	5	–
Ave.		84	9	0

Temperature, shading, and photoperiod effects.—Cowpea plants were kept for 1 wk. at 16°, 20°, 24°, and 28° C. prior to inoculation and were maintained at the same temperatures after inoculation. Growth was poor at 16° but some lesions did develop at that temperature. The number of lesions increased somewhat as the temperature increased but little consistent difference was noted between 20°, 24°, and 28°. Several investigators, working with different viruses, have reported preinoculation shading to be an important factor in increasing the susceptibility of local-lesion hosts (1, 10). Cowpea plants were placed under a layer of cheesecloth for 1 wk. prior to inoculation. In 10 trials, the average number of lesions on shaded plants was 32 while the average on comparable unshaded plants was 28. Plants were tested after being grown for 1 wk. with only a 6-hr. photoperiod per day. In 8 trials the lesion average for the 6-hr. day plants was 46 while the unshaded control average was 35. Although both of the last 2 techniques seemed to increase lesion numbers slightly, neither was helpful enough to improve the value of the cowpea plant as an assay host.

Preinoculation darkening of test plant.—Variation of the factors already discussed failed to produce as large numbers of local lesions as desired. In a further search for an environmental factor which would increase the number of lesions per leaf, preinoculation darkening of test plants was studied. This was suggested by the work of Samuel and Bald (9) with tobacco mosaic virus on *Nicotiana glutinosa* and of Bawden and Roberts (2) with tobacco necrosis virus on French bean and tobacco and with tobacco mosaic and

tomato bushy stunt viruses on *N. glutinosa*. Cowpea plants were kept in continual darkness for 24, 36, and 48 hr. prior to inoculation. Inoculations were made immediately upon exposure to the light. As shown by Table 3, 24- and 36-hr. preinoculation darkening of the test plants resulted in 2 to 3 times as many lesions as appeared in the uncovered controls. Twenty-four hr. of darkening gave the best results, while at 36 hr. the plants were more sensitive to rubbing damage during inoculation and gave fewer lesions. At 48 hr. the leaf tissue was damaged before inoculation, sometimes beyond recovery. In many other trials over a period of more than 2 years plants covered for 24 hr. gave an average of 2.36 times as many lesions per leaf as the controls. Preinoculation darkening was regarded as the most important factor studied in facilitating the production of high lesion numbers for assay purposes.

TABLE 3.—*Effect of preinoculation darkening upon the number of local lesions produced on cowpea when inoculated with cucumber virus 1*

	Average lesions per leaf on plants darkened for period indicated					
	24 hr.		36 hr.		48 hr.	
Trial No.	Darkened	Control	Darkened	Control	Darkened	Control
1	35	0	96	40	149	30
2	204	69	92	39	53	55
3	186	80	139	89	75	100
4	82	58			19	16
5	122	41			46	71
6	81	5			72	17
7	29	6			71	146
8	126	36			13	25
Ave.	108	36	109	56	62	58

SUMMARY AND CONCLUSIONS

A study was made to determine the relative importance of various environmental factors and techniques employed in the use of cowpea as a local-lesion host for the assay of cucumber virus 1. Tobacco plants inoculated in the 3- to 4-leaf stage, grown at 20° to 25° C., and used within 1 wk. after the appearance of conspicuous symptoms provided the best source of the virus. Opposite primary leaves gave nearly identical reaction and provided a better assay than the half-leaf method. A circular motion of a glass spatula with the aid of carborundum was selected as a satisfactory method of inoculation since it gave as high lesion counts as, and less plant injury than, other methods. Primary leaves of plants 12 to 15 days old gave the highest number of lesions and the number declined as the age of the plants increased. Trifoliate leaves gave few or no lesions. Host nutrition had little influence on the number of lesions. Plants growing at 16° C. showed few lesions, and in those grown at 20°, 24°, and 28° the number, though larger, was still not usually as high as desired. Shading of plants with cheesecloth for 7 days and growing at 6-hr. day length before inoculation did not change the lesion number consistently. Preinoculation darkening of the plants proved to be the best procedure to bring the local-lesion number to a high level. When 24-hr., 36-hr., and 48-hr. preinoculation periods were compared, the first period yielded the highest lesion number. The 36-hr. period predisposed the leaves to excessive inoculation injury, and at the 48-hr. period leaves were sometimes injured beyond recovery before inoculation.

Infected cucumber plants were found to be unreliable as a virus source for the assay of strains of cucumber virus 1, because of the presence of an inhibitive substance in cucumber extracts. Further studies of the inhibitor will be presented in a subsequent paper.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. BAWDEN, F. C., AND F. M. ROBERTS. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. **34**: 286-296. 1947.
2. ———, AND ———. Photosynthesis and predisposition of plants to infection with certain viruses. Ann. Appl. Biol. **35**: 418-428. 1948.
3. CHAMBERLAIN, E. E. Cucumber-mosaic (Cucumis virus 1 of Smith, 1937). New Zeal. Dept. Agr. Plant Dis. Div. Bul. 29. 1939.
4. KUNTZ, J. E., AND J. C. WALKER. Virus inhibition by extracts of spinach. Phytopath. **37**: 561-579. 1947.
5. MCKINNEY, H. H. Stability of labile viruses in desiccated tissue. Phytopath. **37**: 139-142. 1947.
6. POUND, G. S., AND J. C. WALKER. Strains of cucumber-mosaic virus pathogenic on crucifers. Jour. Agr. Res. [U. S.] **77**: 1-12. 1948.
7. PRICE, W. C. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. **24**: 743-761. 1934.
8. ROBERTS, DANIEL A., R. E. WILKINSON AND A. FRANK ROSS. Chenopodium hybridum, a local-lesion host for cucumber-mosaic virus. (Abst.) Phytopath. **41**: 31. 1951.
9. SAMUEL, G., AND J. G. BALD. On the use of the primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70-99. 1933.
10. ———, R. J. BEST, AND J. G. BALD. Further studies on quantitative methods with two plant viruses. Ann. Appl. Biol. **22**: 508-524. 1935.
11. WELLMAN, F. L. Identification of celery virus 1, the cause of southern celery mosaic. Phytopath. **24**: 695-725. 1934.
12. ———. The host range of the southern celery-mosaic virus. Phytopath. **25**: 377-404. 1935.

EFFECT OF CERTAIN ANTIBIOTICS ON LESION PRODUCTION BY TWO PLANT VIRUSES [1]

Curt Leben and R. W. Fulton

Plant pathologists have demonstrated increasing interest in the effects of various types of chemicals on inhibition of plant virus infection. Investigations on the subject go back to the work of Duggar and Armstrong (1) in 1925, who reported that sap of certain plants inactivated tobacco mosaic virus when mixed with it prior to inoculation. Since then there have been many reports of similar *in vitro* studies, and a large number of materials are now known to possess inhibitory properties of varying degree. The effect of most, or all, of these substances, however, is observed only when they are mixed with the virus prior to inoculation or when they are applied to leaves within a short time before or after inoculation. This type of virus inhibitor seems to interfere in some way with the process of infection, but an effect on virus multiplication subsequent to infection has not been demonstrated.

In other types of experiments, the test chemical has been applied to the host tissue rather than to the inoculum, often in a manner that prevented direct contact between the inoculum and the test chemical. In these *in vivo* experiments, chemicals of various sorts have been demonstrated to affect infection, symptom development, or virus increase within the host (e.g., 2, 3, 4, 5, 6, 9, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21). The present paper deals primarily with *in vivo* testing and with results that were obtained using antibiotics, azide, and cyanide as test substances. Part of this work has been reported briefly (7).

THE AGAR PLATE TEST.—An agar plate test was devised to determine quickly the effect of various chemicals on development of necrotic lesions. In brief the method consists of placing inoculated, detached leaves on a suitable agar medium in plates and incubating the plates until local lesions appear. Substances to be tested for possible inhibitory effect on lesion development are incorporated in the medium.

Groundwork studies.— Early tests were made by floating leaves on a liquid medium, a method that has been used extensively in detached leaf studies (22). It was found, however, that a semi-solid agar medium possessed advantages over a liquid medium in that 1) the inevitable contaminations were usually minor and localized, and 2) the inoculated surface of the leaves did not accidentally contact the test substance, thus insuring that observed effects were due to the substance moving into the leaf tissue from below.

A number of host-virus combinations yielding local lesions were examined to find those most suitable for our purposes and facilities. Those studied were: cucumber mosaic, tobacco necrosis, and tobacco ringspot viruses on the primary leaves of cowpea (*Vigna sinensis* Endl. var. Blackeye); tobacco mosaic virus on a *Nicotiana* hybrid (*N. tabacum* L. × *N. glutinosa* L.), *N. glutinosa*, and *N. stoctonii* Brandeg.; and 2 strains of tobacco mosaic virus necrotic on *N. sylvestris* Speg. and Comes and *N. plumbaginifolia* Viv. The medium for these tests contained 40 gm. of glucose and 10 gm. of agar per l. Only the upper surfaces of the leaves were inoculated. The lower surfaces, those in contact with the agar, were rubbed with carborundum. Leaves were incubated on the agar in the dark for 4 days at 22–24° C. before data were taken. Lesions were produced in all combinations. With tobacco mosaic virus and the *Nicotiana* hybrid, however, lesion production was not consistent at different times. Further tests with the 3 viruses on cowpeas were also made without rubbing the lower surface of leaves, and lesions were produced by all 3. Tobacco necrosis and tobacco ringspot viruses were selected for the remainder of the work, since these viruses and cowpea seemed particularly suitable.

In searching for a basal medium suitable for development of lesions with the 2 viruses on cowpea leaves a series of tests was run to determine the influence of glucose content and hydrogen-ion concentration of the medium. Glucose was added at 0, 2.5, 5.0, 10.0, 20.0, or 40.0 gm. per l. to a medium containing 10.0 gm. of agar per l. In 2 series of tests no lesions were incited by either virus unless glucose was present. At 2.5 and 5.0 gm. per l. lesions were small and indistinct; at higher concentrations they were distinct, those at 40.0 gm. per l. having somewhat sharper margins. The effect of pH was determined by using a medium containing 10 gm. of agar and 40 gm. of glucose per l. The pH of the medium was adjusted with NaOH or H_2SO_4 to 6 pH levels at and between 3.0 and 7.6. Lesion development of both viruses was normal with media more alkaline than pH 4.0. At pH 3.0, however, no lesions were produced by either virus. Well-developed lesions were obtained with the addition of 4.5 gm. of KH_2PO_4 per l. The basal medium finally adopted, therefore, contained (per liter), glucose 40 gm., agar 10 gm., and KH_2PO_4 4.5 gm. The final pH of media containing various test substances was between pH 4.2 and 5.5.

Methods.—Use of cowpea was advantageous in that plants could be grown quickly under easily standardized conditions. Seeds were planted in vermiculite in shallow trays placed about 30 cm. below 2 white fluorescent lamps (40 watt) that were automatically controlled to give the plants a 14-hr. day. Plants were watered through absorbent cotton wicks with one end buried in the vermiculite and the other extending

[1] Accepted for publication March 12, 1952.

Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

into water reservoirs beneath the trays. The windowless room where plants were grown was kept at 25–27°.

Plants 10–14 days old were used. The upper surfaces of primary leaves were sprinkled with carborundum, wiped lightly with a small gauze pad dipped in inoculum, and washed with tap water. The midrib of each was removed, and the resulting half-leaves were placed with the lower surfaces in contact with the agar test medium.

Tobacco ringspot virus inoculum was prepared by extracting juice from infected young tobacco plants and diluting 1:20 with 0.1 M phosphate buffer at pH 7. Tobacco necrosis virus inoculum was prepared from infected tobacco leaves that had been desiccated at about 1° over $CaCl_2$ and finely ground. About 0.1 gm. of this material was thoroughly macerated in a small quantity of buffer and then buffer added to make 10 ml.

The test substance, which had been treated with a small amount of ethanol to reduce contamination[2], was added after the basal medium had been autoclaved and cooled to 40–50°, mixed well and poured into sterile plates to solidify.

The large plates used in most of the studies were made by covering inverted rimmed lids from glass refrigerator dishes with flat pieces of glass. Plates were approximately 1 × 22 × 22 cm., and 150 ml. of medium was used for each. For each virus 6 half-leaves were usually placed in each plate. These were then incubated 3 or 4 days in the dark at 22–24° before data were recorded.

Preliminary experiments, particularly with low concentrations of glucose and weakly inhibiting concentrations of KCN, showed that partial inhibition was not evidenced by reduction in numbers of lesions formed, but rather by decrease in their size or distinctness. Consequently, data were recorded by rating the degree of lesion development instead of by lesion counts. Each time an experiment was set up lesions on control leaves that had been placed on the basal medium were compared with those on leaves on the test media. Virus concentration of each inoculum was sufficiently high to produce, on control leaves, lesions nearly covering the entire leaf surface.

Results.—A number of substances, mostly antibiotics[3], were examined for their effect on lesion development by the tobacco necrosis and tobacco ringspot viruses. Maximum concentration used for the antibiotics was 1 mg. per ml. Lower concentrations of 3, antimycin A, helixin, and penicillin, were used because greater amounts were not available. Tests with neomycin are reported at 0.5 mg. per ml., since at 1 mg. per ml. leaf contortion was excessive. Results of the tests are in Table 1.

TABLE 1.—*Effects of various substances on lesion development of tobacco necrosis and tobacco ringspot viruses on cowpea leaves*

Basal medium plus:	Concentration	Phytotoxic effects	Lesion development, both viruses[a]
	Mg. per ml.		
Antimycin A	0.02	None	++++
Bacitracin	1.0	Leaf veins blackened	++++
Chloromycetin	1.0	Do	++++
Helixin[b]	0.02	None	++++
Neomycin	0.5	Leaves water congested	++++
Penicillin G, K salt	0.37	None	++++
Potassium cyanide	0.65	Leaves flaccid, yellowed	—
Do	0.065	Do	++
Sodium azide	0.65	Do	—
Do	0.065	Do	—
Streptomycin sulfate	1.0	None	++++
Streptothricin hydrochloride	1.0	Leaves yellowed in 2 of 3 tests	—[d]
Do	0.5	None	++[c]
Terramycin hydrochloride	1.0	Do	—[d]
Do	0.5	Do	++[c]
Basal medium only	....	Do	++++

[a] ++++ = Normal lesion development; — = no symptoms. Results based on 2 tests, except as noted.
[b] See reference 8. A partly purified preparation containing 2.5 units per mg. was used. In 1 additional test no phytotoxic effects or inhibition of symptom development was noted at 0.6 mg. per ml.
[c] Result based on 1 test.
[d] Result based on 3 tests.

Of the 11 substances, streptothricin, terramycin, KCN, and NaN_3 prevented lesion production. Phytotoxic effects were produced by many of the test materials, including 3 of those that inhibited lesion production.

IN VITRO TESTS.—Since streptothricin, terramycin, KCN, and NaN_3 inhibited symptom development, it seemed of interest to determine whether this inhibitory effect could be attributed to direct action on the virus as determined by *in vitro* tests. A series of tests was run, using primary leaves of cowpea plants grown in the greenhouse. Virus inocula, prepared with 0.1 M phosphate buffer at pH 7, and test substances, both at twice the desired final concentrations, were mixed in equal amounts. The mixtures were allowed to stand for 1–2 min. and then inoculated, with gauze pads, on halves of 8 primary leaves dusted with carborundum. The opposite half-leaves were treated in the same way with the virus inoculum diluted with an equal volume of water.

[2] The final concentration of ethanol was 3.3 ml. per l. In 2 preliminary experiments, concentrations as great as 10 ml. per l. had no effect on symptom development of either virus.

[3] We are indebted to the following concerns for generous samples of antibiotics: Commercial Solvents Corporation (bacitracin, neomycin, and penicillin); Bristol Laboratories, Inc. (penicillin and streptomycin); Eli Lilly and Co. (streptomycin); Merck and Co., Inc. (streptothricin); Parke, Davis and Co. (chloromycetin); Chas. Pfizer and Co. (terramycin).

Results with tobacco necrosis virus.—Two *in vitro* tests were made with the same sample of streptothricin used in the agar plate experiments, 1 test at 0.5 mg. per ml. and the other at 1.0 mg. per ml. Lesion counts were (treated/control), respectively, 606/515 and 348/425, indicating that the antibiotic did not inactivate the virus, a finding previously reported (7). In 2 later tests with a second sample of streptothricin at 1.0 mg. per ml. counts of 93/750 and 60/355 were obtained, indicating that this sample had some deleterious effect on infectivity. Two trials with terramycin at 1.0 mg. per ml. gave lesion counts of 413/541 and 1148/1270. Potassium cyanide, tested at 0.65 and 1.0 mg. per ml., gave lesion counts of 1170/1101 and 1284/1341, respectively. Sodium azide was tested at the same concentrations, and the lesion counts were, respectively, 996/861 and 908/1555.

Results with tobacco ringspot virus.—Tests with streptothricin were made at 0.5 and 1.0 mg. per ml., and the number of lesions produced were (treated/control) 132/112 and 91/84, respectively. In a third test, using the second sample of streptothricin at 1.0 mg. per ml., the lesion counts were 622/704. Terramycin was tested twice at 1.0 mg. per ml., the lesion counts being 83/86 and 741/734. Lesion counts for 0.65 mg. per ml. of potassium cyanide in 3 tests were 79/14, 474/417, and 851/869. Counts for sodium azide at the same concentration were 36/45, 330/368, and 901/879.

These results indicate that the 4 substances found to be effective in preventing lesion development in agar plate tests had no marked inhibitory action *in vitro* on either of the 2 viruses, except that 1 sample of streptothricin appeared to inactivate tobacco necrosis virus.

Inhibition of tobacco mosaic virus.—The observation that inhibitory compounds reduced the degree of lesion development rather than lesion numbers in agar plate tests suggested that virus multiplication was inhibited after infection had taken place. To examine this point and to extend trials to another virus, several tests were made to determine the effect of terramycin on tobacco mosaic virus. *N. tabacum* var. Havana Seed leaves were heavily inoculated, detached, and half of each leaf placed on the basal medium described above. The opposite half was placed on basal medium plus 0.5 mg. per ml. of terramycin. Five disks 4 mm. in diameter were taken from leaf-halves at various times and frozen immediately. About 12 hr. after the last sample had been taken each set of 5 disks was ground in 1 ml. of 0.1 M phosphate buffer at pH 7. These suspensions were further diluted 1:50 with buffer and the infectivity compared by inoculating to 4 leaves of 4 *Nicotiana* plants in a latin square arrangement, with "terramycin" and "control" samples on opposite half-leaves. In each of 3 trials, concentration of the virus, as indicated by this infectivity test, was lower in leaves on the medium containing terramycin (Figure 1).

In other trials heavily inoculated tobacco leaves were placed for the first 24 hr. on basal medium and then for 24 hr. on basal medium plus terramycin, samples being taken every 12 hr. The terramycin concentration was 0.5 mg. per ml. in 1 test, and 1.0 mg. per ml. in another. No inhibitory effect was noted. In other experiments inoculated leaves were placed on terramycin medium for 36 hr. and then transferred to basal medium for 24 hr. Samples were taken at 24, 36, 48, and 60 hr. from the time of inoculation. In each of 2 trials, 1 at 0.5 and 1 at 1.0 mg. of terramycin per ml., the decrease in virus concentration induced by terramycin continued to be observed for 12 and 24 hr. after leaves had been removed from the terramycin agar, although during this time the virus continued to multiply.

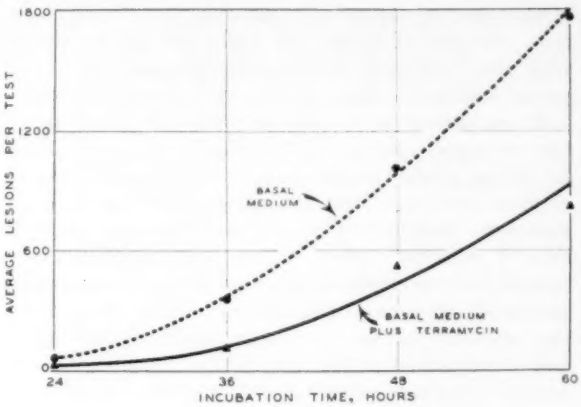


Fig. 1—Concentration of tobacco mosaic virus in detached tobacco leaves incubated on basal medium and on basal medium plus terramycin for various times, as indicated by local lesion assays. Each point is the average of 3 tests.

These data suggest that tobacco mosaic virus multiplication was inhibited by terramycin. There also exists some possibility that terramycin influenced the infection process rather than virus multiplication, since the amount of virus in leaves placed on basal medium for 24 hr. and then on terramycin medium for 24 hr. was not affected by the chemical. It is probable, however, that infection was completed between the time the leaves were inoculated and when they were placed on the agar (15-30 min.) and before the terramycin had passed through the leaf and reached the upper epidermal areas.

Discussion.—The present report appears to be the first to describe plant virus inhibition by antibiotics. Manil (10) was unable to prevent infection with tobacco mosaic virus by application of actinomycetin, actinomycin, penicillin, streptomycin, or tyrothricin to the host leaves or to the inoculum, or by allowing plants to absorb the antibiotics. Weindling et al. (18) have referred to the paper in press by Beale and Jones in which there was shown to be no inhibition of tobacco mosaic or potato yellow dwarf viruses by aureomycin, chloromycetin, penicillin, streptomycin, or terramycin when these materials were used systemically

in the plant or mixed with inoculum prior to inoculation. Results of Beale and Jones are not in accord with those reported here, since we found that terramycin inhibited tobacco mosaic virus multiplication.

It was shown in the present work that it was necessary to supply glucose to detached leaves in the dark for lesion production by tobacco necrosis and tobacco ringspot viruses. This finding suggests that leaf tissues were deficient in usable carbohydrates and that once a suitable carbon source was supplied, more normal host metabolism was resumed and lesions were produced. In view of the requirement for glucose and the inhibitory action of cyanide and azide, which are both inhibitors of certain respiratory enzymes, it seems possible that multiplication and symptom development of tobacco necrosis and tobacco ringspot viruses are dependent on plant respiration. This view has been expressed previously by Woods (19) and by Woods and DuBuy (21). Cyanide was reported by these workers to inhibit tobacco mosaic virus (19, 20, 21) and tobacco ringspot virus (21) multiplication as determined by infectivity tests and to inhibit reversibly lesion development by these 2 viruses (19, 21). Kalmus and Kassanis (5) have reported that CO_2 reduced susceptibility of beans to tobacco necrosis viruses, and, noting the work of Woods, suggested that the effects of cyanide and CO_2 may be linked, since CO_2 reversibly inhibits plant respiratory systems.

Association of virus increase with host metabolism is also indicated by the finding that 3 of the 4 compounds that were inhibitory *in vivo* had little or no effect when tested *in vitro* at the same concentration. The actual concentration of these materials in the leaf is not known, but it seems likely that it was no greater than in the *in vitro* tests. Inhibitory action *in vivo* without *in vitro* action has also been described by Kalmus and Kassanis for CO_2 (5), by Gendron for growth products of a microorganism (2), by Matthews for guanazolo (11), and by Takahashi for malachite green (17).

The agar plate test described in this paper appears to offer a convenient and simple way to screen materials for plant virus inhibitors. It is noteworthy that the compounds that inhibited lesion production did so with both viruses, and that 1 of these compounds, terramycin, inhibited the increase of a third virus in a different host. Since the materials must enter the leaf to inhibit the virus, those that are effective possess at least limited systemic properties. In the agar plate test some assessment of the phytotoxicity of the test material may be made, and it seems significant that some of the antibiotics examined were markedly phytotoxic, but had no virus inhibiting action (Table 1). The test also appears to be of interest because with at least 2 viruses it offers a ready means for investigation of the influence of host metabolism on virus increase through studies with metabolites, metabolic inhibitors, and substances of significance in the physiology of the plant.

SUMMARY

An agar plate method for determining the inhibitory action of test substances on lesion production by plant viruses was developed. Detached leaves were placed, inoculated side uppermost, on an agar medium containing the test substance and incubated in the dark. It was necessary to add glucose to the agar for lesion formation by tobacco necrosis and tobacco ringspot viruses on cowpea leaves. Of 9 antibiotics tested for inhibitory action with these host-virus combinations, 2, streptothricin and terramycin, prevented lesion production, as did KCN and NaN_3. Terramycin, KCN, and NaN_3 did not reduce infectivity of either virus when mixed with the inoculum prior to inoculation. Neither of 2 samples of streptothricin altered infectivity of tobacco ringspot virus, but 1 of the 2 partly inactivated tobacco necrosis virus. The rate of tobacco mosaic virus increase in detached tobacco leaves, as indicated by local lesion assay, was less in leaves on a medium containing terramycin than in leaves on a control medium.

Departments of Plant Pathology and Horticulture
University of Wisconsin
Madison, Wisconsin
and
Division of Tobacco, Medicinal and Special Crops
U. S. Department of Agriculture

LITERATURE CITED

1. Duggar, B. M., and Joanne Karrer Armstrong. The effect of treating the virus of tobacco mosaic with the juices of various plants. Ann. Mo. Bot. Gard. **12**: 359-366. 1925.
2. Gendron, Yves. Action des produits du métabolisme d'un champignon sur la multiplication du virus de la mosaique du tabac et du virus X de la pomme de terre chez le tabac. Acad. Sci. Compt. Rend. **230**: 1890-1891. 1950.
3. Hartman, Richard T., and W. C. Price. Synergistic effect of plant growth substances and southern bean mosaic virus. Amer. Jour. Bot. **37**: 820-828. 1950.
4. Howles, R. Virus diseases. Rept. Expt. Res. Sta. Cheshunt 1950 **36**: 26-34. 1951.
5. Kalmus, H., and B. Kassanis. Reduction by carbon dioxide of susceptibility of beans to tobacco necrosis viruses. Nature **154**: 641-642. 1944.
6. Kutsky, R. J., and T. E. Rawlins. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by a spectrophotometric method. Jour. Bact. **60**: 763-766. 1950.
7. Leben, Curt, and R. W. Fulton. The inhibition of virus symptom expression by sodium azide, potassium cyanide, and two antibiotics. (Abstr.) Phytopath. **41**: 23. 1951.
8. ———, G. J. Stessel, and G. W. Keitt. Helixin, an antibiotic active against certain fungi and bacteria. Mycologia (In press.)
9. Lock, Seth B. Studies on the chemotherapy of potato virus diseases. (Abstr.) Phytopath. **38**: 916. 1948.
10. Manil, P. Action négative d'antibiotiques sur des virus phytopathogènes. Proc. 4th Internat. Congr. Microbiol. 1947. Rosenkilde and Bagger. Copenhagen. 1949.
11. Matthews, R. E. F. Effect of some substituted purines

on the development of plant virus infections. Nature **167**: 892-893. 1951.
12. RIJKOV, V. L., AND B. A. SMIRNOVA. The influence of cations on the necrotic reaction of Nicotiana glutinosa. Mikrobiologiia **17**: 96-99. 1949. (Biol. Abstr. **23**: 30299. 1949.)
13. RIPPEL, KARL. Die Inaktivierung des Blattrollvirus durch Nucleinsäure und die Überführung des Blattrollvirus in Kraüselmosaik (A-virus) durch ein proteolytisches Ferment bei Solanum tuberosum. Naturwiss. **36**: 188. 1949. (Biol. Abstr. **25**: 6341. 1951.)
14. RUMLEY, GAIL E., AND W. D. THOMAS, JR. The inactivation of the carnation-mosaic virus. Phytopath. **41**: 301-303. 1951.
15. STODDARD, ERNEST M. The X disease of peach and its chemotherapy. Conn. Agr. Expt. Sta. Bull. 506. 19 pp. 1947.
16. ———, AND A. E. DIMOND. Malachite green suppresses symptom expression of tobacco mosaic virus in tomato. (Abstr.) Phytopath. **39**: 24. 1949.
17. TAKAHASHI, WILLIAM N. The inhibition of virus increase by malachite green. Science **107**: 226. 1948.
18. WEINDLING, R.; H. KATZNELSON, AND HELEN PURDY BEALE. Antibiosis in relation to plant diseases. Ann. Rev. Microbiol. **4**: 247-260. 1950.
19. WOODS, MARK W. Reversible inhibition of tobacco mosaic virus in living cells with 0.0002 molar sodium cyanide. Science **91**: 295-296. 1940.
20. ———. Effect of cyanide on synthesis of ring-spot and mosaic viruses in tobacco. Phytopath. **33**: 77-80. 1943.
21. ———, AND H. G. DUBUY. The effect of tobacco-mosaic virus on cellular respiration. Phytopath. **32**: 288-302. 1942.
22. YARWOOD, C. E. Detached leaf culture. Bot. Rev. **12**: 1-56. 1946.

CANTALOUPE POWDERY MILDEW CONTROL WITH DINITRO CAPRYL PHENYL CROTONATE [1]

G. H. Godfrey

Sulphur, a standard remedy for powdery mildew on many crops, can not be used safely on cantaloupes except for 1 or 2 sulphur-resistant varieties. Copper fungicides and most organic fungicides do not adequately control powdery mildew (*Erysiphe cichoracearum* DC.) on cantaloupes, or are otherwise undesirable.

Downy mildew (*Pseudoperonospora cubensis* [Berk. and Curt] Rostow.) has been the chief limiting factor in cantaloupe production in the Lower Rio Grande Valley and some other areas of Texas. Unfortunately, most powdery-mildew-resistant varieties are susceptible to downy mildew, and are therefore not entirely suitable for planting in these areas. Conversely, most of the downy-mildew-resistant varieties are susceptible to powdery mildew, and this has left the powdery mildew problem unsolved for these sections of Texas.

Experiments conducted in 1950 and 1951 have shown that a new organic fungicide, dinitro capryl phenyl crotonate,[2] is effective in checking the disease without injury to the plants.

LITERATURE REVIEW.—Dinitro capryl phenyl crotonate was first used as an acaricide, though some of the early work indicated fungicidal as well as insecticidal value. Lathrop and Hilborn (4) report good control of European red mite on apples in Maine, and also state that leaf and fruit scab were held under very satisfactory control. Hofmaster and Greenwood (3) report this material to be one of the best for the control of two-spotted mite in strawberries in Virginia, with good rapid action and good lasting properties. Wene and Otey (7) report that Karathane (1 per cent active) gave good control of red spiders in celery, with good residual effectiveness. Fair control was also obtained in cucumbers.

Use of dinitro capryl phenyl crotonate as a fungicide has also received some attention. Yarwood (9) reported that at 0.002 per cent it gave 95 per cent control of 5-day-old bean mildew infections and at 0.003 per cent protected plants from infection. It was the most effective of all fungicides tested. Yarwood (10) also stated that for situations where sulphur dust is objectionable the fungicide is potentially the most useful one for powdery mildew control yet tested. Arathane (1) was superior to lime-sulphur in controlling apple powdery mildew in California, and only slightly less effective than lime-sulphur in controlling peach powdery mildew. It was not injurious to peach foliage whereas lime-sulphur was. It did not control brown rot spur blight of cherries or black rot of grapes. According to Rich and Horsfall (6) it is an effective acaricide which also controlled potato late blight in the 1945 insecticide field trials of Dr. F. B. Maughan. They also report that 2 lb. per 100 gal. as a spray gave fair control of bean anthracnose, Septoria and Cercospora blights of celery, and good control of early apple scab. Wilson (8) lists it among several materials that did not produce significant mortality of conidia of *Sclerotinia laxa*. McClellan and Smith (5) report promising results from the use of Karathane spray at ½ lb. per 100 gal. in controlling powdery mildew and 2-spotted spider mites on roses.

OBSERVATIONAL TESTS.—Exploratory tests were conducted in March, 1950, with sprays and dusts made from wettable Karathane concentrate containing 25 per cent dinitro capryl phenyl crotonate. Spray applications were made with 1.25 lb. of the concentrate containing 0.31 lb. of the active ingredient to 100 gal. of water. When used as a dust, 4 lb. of concentrate

[1] Accepted for publication March 11, 1952.

[2] Dinitro capryl phenyl crotonate was originally manufactured by Rohm and Haas Company of Philadelphia as Arathane and later as Karathane. Recently the same material has been taken over by Innis, Speiden & Co., Inc., of New York and sold as Iscothan.

[3] In a personal communication Dr. Yarwood called the writer's attention to a case of severe injury on peaches in the Stockton, California area, apparently caused by Karathane.

were mixed with 96 lb. of inert carrier to give a 1 per cent mixture.

When the applications were thorough, both the spray and the dust killed the fungus in mildewed spots on the leaves. No increase in size of such mildew spots was evident a week after treatment and no injury to plants was apparent. With adjacent non-treated plants badly affected, mildew began to appear again on treated plants 2 wk. after treatment. This infection was mostly on new leaves. Another thorough dust application was made, covering the former check plants as well. Further spread of mildew was completely stopped.

Additional observational tests were conducted with 1 per cent dust, beginning in late March when fruits were just beginning to set. Applications were made on portions of 10 large commercial cantaloupe fields upon which powdery mildew was beginning to appear. Karathane was not available in quantities sufficient to cover the entire acreages, so only small blocks in each field were treated. Large areas of non-treated plants were available in each case for comparison. Results suggested that Karathane offers promise for powdery mildew control in cantaloupes. The following observations were made:

(1) The material acted as an eradicant fungicide, killing both mycelium and spores of the fungus. Microscopic examination showed spores and hyphae to be shrivelled. No increase in size of mildew spots occurred. Non-treated spots of infection continued to increase in size and to produce spores.

(2) Plants were not injured by the fungicide.

(3) A single thorough application made when mildew was just beginning to appear in the field controlled the pathogen sufficiently to keep plants green for several weeks. In a specific case, with duplicate plots, plants were green 40 days after application. Only 10 to 15 per cent of the leaves showed mildew, which was mostly on new leaves, and only occasional leaves died. In 2 comparable check blocks, 40 to 50 per cent of the leaves were off-color, due to heavy infection by powdery mildew, and about 15 per cent died.

(4) Effectiveness of an application was, in general, proportional to the rate of application. Careful hand applications at 40 lb. per acre, aimed to reach the lower as well as the upper surfaces of leaves, gave good control; airplane applications at 15 lb. per acre gave poor control. Failure to get good control in some cases could be attributed to poor leaf-surface coverage either from poor application or from too low rate of application.

(5) Thorough coverage of lower leaf surface appeared to bring about better disease control than that obtained by application in a downward direction only.

(6) Effectiveness of treatment in keeping plants green was proportional to number of applications. In a specific case, plants given 4 applications at about weekly intervals had a healthy green color at the beginning of harvest, those given 3 applications had considerable off-color foliage, while non-treated plants were brown and non-productive of marketable melons.

(7) Timing of applications in relation to rain appeared to be important. In a specific case plants dusted the second day after a ¼ inch rain were green 10 days later while adjacent plants dusted the fourth day after the rain were brownish.

RANDOMIZED PLOT EXPERIMENTS.—*Experiment 1.*—A randomized plot dusting experiment was started on a late-planted field on May 18, 1950, just before the first harvest. Powdery mildew infection was light but fairly uniform throughout the field. Three rates of application of 1 per cent dinitro capryl phenyl crotonate dust were used: light, medium and heavy, as shown in Table 1, and a check was left untreated. There were 4 replications of each treatment. Three applications were made at 9-day intervals. The methods used in recording and analyzing the data from the experiment are similar to those used by Heuberger (2). Damage from powdery mildew was estimated at the close of the test, a week after the final application, on a scale of index numbers from 0 to 10. Index number 10 indicated that all leaves were killed, the numbers then grading downward to 0 on the basis of the proportion of leaves obviously damaged as manifested by yellow to brownish color. Results are in Table 1.

TABLE 1.—*Cantaloupe powdery mildew control with 1 per cent dinitro capryl phenyl crotonate dust at different rates of application*

Treatment lbs. per acre	Disease index				
	1	2	3	4	Mean
20 to 25	7	8	7	8	7.50
30 to 35	5	7	7	6	6.25
45 to 50	5	6	5	5	5.25
0	9	10	10	10	9.75

LSD at 5 per cent level, 0.77; at 1% level, 1.105.

The data show that dinitro capryl phenyl crotonate was effective in reducing powdery mildew in cantaloupes and that a heavy application was more effective than a lighter one. Appearance of mildew spots on new leaves, evident at time of the second and third dust applications, suggested that the interval between applications was too long. This was particularly true in this experimental area in which the non-treated plants had quickly become heavily infected and were a constant source of new infection.

Experiment 2.—A small randomized plot test was conducted in the fall of 1951. The experimental area was a small field of powdery-mildew-susceptible cantaloupes. It lay adjacent to a field of summer squash heavily infected with powdery mildew, and on the leeward side with relation to prevailing winds. Powdery mildew was just beginning to appear on the cantaloupes. Ten-hill plots were used, and there were 5 replications of treatments and checks. In one treatment (designated "single" in Table 2) a light application of dust was applied by means of a rotary hand duster with the nozzle held close to the ground. The dust was blown through the plants and some of the

excess was carried back through and over the plants by the gentle breeze. The second and more thorough treatment (designated "double") involved turning the vines over in order to insure complete coverage by direct application on mildew spots on lower surfaces of leaves. This was followed immediately by a second light delivery of dust over the tops of the replaced vines. It was recognized that this would not be a practical method for large-scale field operations, but it should aid in determining the value of thorough coverage. Using individual plants, readings were taken 10 days after the treatment in terms of disease index numbers from 0 to 10 based upon percentages of the leaves showing spots. Results are in Table 2.

TABLE 2.—*Cantaloupe powdery mildew control with dinitro capryl phenyl crotonate by different methods and rates of application*

	Average disease index by plots					
	Replication					
Treatment	1	2	3	4	5	Mean
Single (20 pounds)	2.2	.9	.9	2.6	.6	1.44
Double (35 pounds)	.3	.4	.8	.3	.2	.40
Check	6.1	6.1	5.5	6.1	4.8	5.72

LSD at 1 per cent level, .813

The light application showed highly significant disease control, even though recent infections occurring on young terminal leaves were counted as infected. Substantially greater efficiency in disease control was derived from thorough coverage of mildew spots on both leaf surfaces with the fungicidal dust.

DISCUSSION.—An effective reduction of powdery mildew in cantaloupes with dinitro capryl phenyl crotonate in dusts containing 1 per cent of the active ingredient, was obtained without injury to plants. A significant finding was that better mildew control was obtained by thorough coverage of existing mildew spots on all parts of the plant. A practical method of obtaining such coverage with a low-lying vine crop is a problem. One grower used a "skirt" attachment wired onto his duster. The operator directed the dust rearward as he walked forward along the row. Fairly efficient swirling of the dust through the foliage of the plants was obtained through the 8-ft. length and 6-ft. width of the skirt. This device was used regularly by the grower, and the plants were kept alive to harvest stage in a season of exceedingly heavy mildew incidence.

Tests have indicated that this fungicide is efficient in residual, or preventive control. New infections that occurred after a treatment were mostly on newly developed foliage rather than on old leaves which, without treatment, are the first to die. This observation also supports the early finding that this fungicide has good eradicant properties on powdery mildew. Spots of infection that were well covered with dust or spray did not spread or enlarge. The fungus, which became dry and brownish in color, was dead as shown by microscopic examination. Heavy rates of application are desirable for best disease control. Seven-day intervals between applications would appear to be preferable to 9-day or longer intervals, particularly if sources of infection are general. Longer intervals may be satisfactory when the variety is partially resistant or when the season is unfavorable to rapid spread of the disease. Where powdery mildew is a serious factor, treatments should be repeated shortly after a washing rain and should be continued until just before the end of harvest. Since 1 per cent dust has given consistently good results, it may be that a lower concentration would prove satisfactory as reported by McClellan and Smith (5) on roses and by Yarwood (9) on beans. Need for further research along this line is indicated.

SUMMARY

Several preliminary observational tests and two randomized plot experiments have shown that dinitro capryl phenyl crotonate, available now as Iscothan (see footnote on title page), in the form of a dust containing 1 per cent of the active ingredient, is an effective control for powdery mildew in cantaloupes. Light applications (20 to 25 pounds per acre) gave a fair degree of control. Heavier and more thorough applications, which covered lower leaf surfaces as well as upper, gave excellent control. The fungicide was eradicant in its action, killing mycelium as well as spores. It appeared to have good residual properties. In no case was injury to foliage observed.

TEXAS AGRICULTURAL EXPERIMENT STATION
SUBSTATION NO. 15
WESLACO, TEXAS

LITERATURE CITED

1. Fungicide Committee, American Phytopathological Society: Subcommittee on testing and results of newer fungicides. Nation-wide results with fungicides in 1949. U. S. Dept. Agr., Pl. Dis. Rptr. Suppl. 192: 121-187. 1950.
2. HEUBERGER, J. W. Organic and copper fungicides for potatoes and their compatibility with DDT. Del. Agr. Exp. Sta. Bul. 274. 1948.
3. HOFMASTER, R. N. AND D. E. GREENWOOD. Control of the two-spotted mite on strawberries. Jour. Econ. Entom. **44**: 514-519. 1951.
4. LATHROP, F. H. AND M. T. HILBORN. European red mite control. Jour. Econ. Entom. **43**: 172-175. 1950.
5. MCCLELLAN, W. D. AND FLOYD F. SMITH. Karathane looks promising for mildew and spider control. The Florists' Review. Aug. 31, 1950.
6. RICH, SAUL, AND JAMES G. HORSFALL. Fungicidal activity of dinitro capryl phenyl crotonate. (Abstr.) Phytopath. **39**: 19. 1949.
7. WENE, GEORGE P., AND GEORGE OTEY. Control of red spider mites on vegetables. Rio Grande Valley Horticultural Inst. Proc. **5**: 16-19. 1951.
8. WILSON, E. E. Sodium pentachlorophenate and other materials as eradicative fungicides against Sclerotinia. Phytopath. **40**: 567-583. 1950.
9. YARWOOD, C. E. Fungicides for the control of powdery mildew. Int. Cong. Crop Protection II. Abstracts of Communications, 13-14. 1949.
10. YARWOOD, C. E. Fungicides for powdery mildews. Int. Cong. Crop Protection II. Proc. (Reprint) 1-22. 1949.

The Antibiotic Activity of Thiolutin in the Chemotherapy of the Fusarium Wilt of Tomato. K. S. GOPALKRISHNAN AND J. A. JUMP.[1]—Previous studies by the authors[2] on activity of thiolutin have demonstrated its inhibition of a number of fungus parasites *in vitro* and its comparatively low phytotoxicity. This antifungal antibiotic was isolated by Tanner et al[3] from a strain of *Streptomyces albus.*

The tomato variety, Bonny Best, was selected for the present studies because of its high susceptibility to wilt caused by *Fusarium oxysporium lycopersici.* Young plants were grown from seed in flats of soil and at the 4-leaf stage were removed for experiment, after selection for uniformity. After the root systems had been washed to free them from soil, the plants were transferred to 125 ml. Erlenmeyer flasks containing Knop's solution with various concentrations of thiolutin. They were left in this solution for 4 days.

It had previously been determined on the basis of agar plate assays that growth of the Fusarium would take place in concentrations of thiolutin of at least 100 p.p.m. However, at this high level, growth commenced 8 days later than in the control and proceeded at a much slower rate (Fig. 1). Because of the phytotoxicity of high levels of thiolutin, solutions in which the tomato plants were treated were adjusted to 10, 20, 40, and 80 p.p.m.

After the antibiotic treatment, the root systems were washed and injured by trimming off tips of a few roots of each plant. Roots were then immersed for 10 min. in a suspension of the *Fusarium* at a concentration of more than a million spores per ml. The plants were then transferred to pots of sand and watered as necessary with Knop's solution. A second inoculation was made 2 days later by watering the plants with a heavy spore suspension. Eight plants were used for each treatment and for the inoculated untreated control.

Typical wilt symptoms first appeared in controls on the sixth day after inoculations. By the eighth day half the controls were completely wilted. The remainder were completely wilted by the twelfth day. All the treated plants remained free from wilt symptoms for the duration of the experiment. These results were confirmed by a second experiment in which 10 plants per series were used. Although the treated plants remained free from disease, those which were treated with the higher concentrations of thiolutin showed a distinct stunting accompanied by marked epinasty of leaves (Fig. 2). There was no significant difference between averages of moist weights of plants of the first 3 series, but the 80 p.p.m. series averaged only 77 per cent of the mean weight of the other series 3 weeks after treatment.

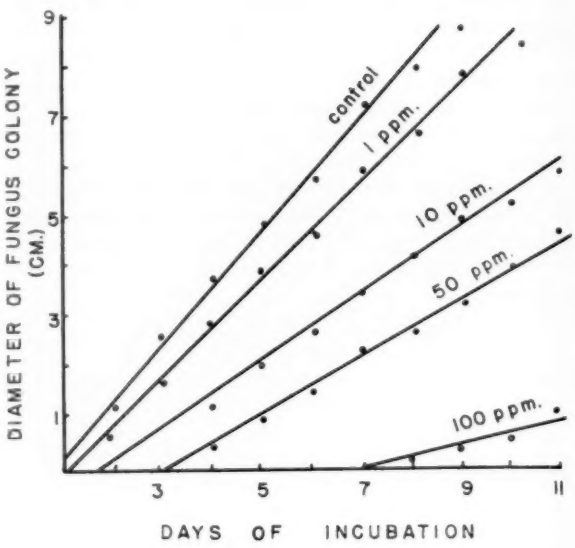


FIG. 1.—Growth of *Fusarium lycopersici* at various concentrations of thiolutin.

An attempt was made to isolate the fungus from the treated and untreated inoculated series. It could be isolated readily from roots and stems of the inoculated untreated controls, occasionally from the 10 and 20 p.p.m. series, and not at all from the 40 and 80 p.p.m. series. Thus, under these particular experimental conditions, thiolutin appears to be more active *in vivo* than *in vitro.* Whether concentration of the antibiotic occurs in the host tissue or whether there may be a synergistic reaction between the antibiotic and some metabolite of the host plant has not been determined with certainty. There is evidence of the latter, since the authors have determined that some amino acids, particularly histidine and methionine, enhance the *in vitro* inhibition of certain fungi by thiolutin.

Thiolutin does not appear to be in the guttation drops of treated plants as Brain et al[4] have reported to be the case in oats treated with griseofulvin. To determine whether thiolutin was excreted in the guttation drops, a series of tomato plants which had been treated with thiolutin by root immersion were kept under bell jars during and subsequent to treatment. The drops were collected and assayed against a stan-

[1] The authors wish to express their appreciation to Chas. Pfizer & Co., Inc. for supplying the thiolutin used in this investigation.

[2] Gopalkrishnan, K. S. and J. A. Jump. The activity of thiolutin against certain phytopathogenic fungi and seed-borne diseases. Proc. Ind. Acad. Sci. 1951 (in press)

[3] Tanner, Fred W., John A. Means, J. W. Davisson, and Arthur R. English. Thiolutin, an antibiotic produced by certain strains of Streptomyces albus. Charles Pfizer and Company, New York (in press).

[4] Brian, P. W., J. M. Wright, J. Stubbs, and A. M. Way. Uptake of antibiotic metabolites of soil microorganisms by plants. Nature 167: 347-349. 1951

FIG. 2.—Tomato plants treated with various concentrations of thiolutin and infected with *Fusarium lycopersici*. Left to right: no thiolutin, 10ppm., 20ppm., 40ppm., 80ppm.

dard thiolutin solution with spores of *Alternaria solani* as the test fungus. No activity could be detected.

Less successful results were obtained when thiolutin chemotherapy was attempted by watering a solution of 40 and 100 p.p.m. directly upon the sand in which the tomatoes were growing. After 4 days of treatment the plants were removed, inoculated as before, and replanted in fresh sand. A few plants remained healthy in the 100 p.p.m. series, and the treated plants exhibited wilting at a later date than the controls. The generally inferior results led to the supposition that the antibiotic was largely adsorbed by the sand medium; so if a soil which contained an appreciable clay fraction would probably not be suited to thiolutin chemotherapy. This seemed to be borne out by a later experiment in which tomato plants growing in pots containing a heavy greenhouse potting soil were watered with thiolutin. The concentration of thiolutin used was calculated to bring the soil solution to a concentration of 40 p.p.m. in one series and 100 p.p.m. in another, assuming no adsorption. All the plants in both treated series wilted, and there was no difference in the time of wilting between treated plants and inoculated controls. A test of the soil extract half an hour after application of the antibiotic showed that the level of activity of the 100 p.p.m. series was less than that of a 10 p.p.m. standard solution. No activity could be detected after 24 hr. The presence of organic matter and microbiological activity may have been a partial factor in the loss of potency of thiolutin in soil, but these experiments would tend to indicate that the presence of a considerable clay fraction was the dominating factor.—University of Notre Dame, Notre Dame, Indiana.

Transmission of a Leaf Mosaic Associated with Color Break in the Flowers of Dendrobium superbum Reichb. f.[1] HARRY H. MURAKISHI. A leaf mosaic and a blossom color break of *Dendrobium superbum*, known in Hawaii as the "Honohono" orchid, have been observed throughout the Territory for many years. Affected leaves have yellowish-green streaks between the veins, while the areas nearest the veins are often a darker green, resulting in alternate short streaks of yellowish-green and green. In some diseased plants, necrotic streaks on leaves often accompany the yellow-green mottling. The mosaic symptoms (Fig. 1) are most pronounced on young shoots. Green areas, especially in older leaves, are thicker than the yellowish-green areas, causing the leaf to be undulating and distorted. Diseased plants are noticeably shorter in leaf and stem lengths when compared to healthy plants. Plants affected with leaf mosaic also produce flowers with breaking in the color of sepals and petals.

Transmission tests to determine the cause of the disorder were made using an inarch-graft method[2] and by juice inoculations. In the first method a tangential cut of 3 to 4 ins. was made on the young stem several ins. below the growing point and the cut surface brought in contact with a similar cut made on a healthy plant. The two stems were bound together at the joined cut surfaces and the union coated with warm melted paraffin. Out of six graft pairings, five formerly healthy plants produced leaf mosaic symptoms. In the control, six similar graft pairings of twelve healthy plants remained symptomless.

In the juice transmission test, eight healthy plants

[1] Published by permission of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 241.

[2] Murakishi, Harry H. Color breaking of Vanda orchids. Pac. Orchid Soc. Bul. 9 (1): 13-16. 1950.

Grateful acknowledgment is made to various members of the Ewa, Honolulu, Pacific, and Waipahu Orchid Societies for their donations of Dendrobium plants.

FIG. 1. Leaf symptoms on naturally infected plant.

were inoculated by rubbing sap from an infected plant into the leaves with the aid of No. 500 carborundum powder. All plants thus inoculated developed leaf mosaic, while seven control plants treated similarly, but with non-infective juice, remained healthy. All plants experimentally infected, whether by inarch grafting or by leaf rubbing, developed mosaic symptoms only on new leaf growth or on side shoots produced after inoculation. Older leaves present at the time of inoculation remained unaffected. Whether the virus causing leaf mosaic is also the cause of blossom color break is as yet undetermined since none of the test plants have flowered.

Time required for the expression of leaf symptoms in graft-inoculated plants ranged from 30 to 116 days, averaging 30 to 45 days. In one of the grafted test plants where the incubation period was 116 days, the rate of new leaf growth development was unusually slow. In the juice transmission test, symptoms appeared uniformly within 30 to 33 days after inoculation.

Nobrega[3] described a leaf mosaic of *Dendrobium nobile* in Brazil but reported no color-break symptoms. This virus was found to be transmissible to *Nicotiana tabacum*, *N. glutinosa*, and *N. rustica* but not to *D. nobile*.

A preliminary host range test with the "Honohono" mosaic virus indicated that *N. tabacum* and *N. rustica* are resistant. Further host range tests and a study of the physical properties are being made in an effort to determine the relationship of the virus to other orchid viruses.—University of Hawaii, Agricultural Experiment Station, Honolulu, Hawaii.

[3] Nobrega, N. R. Uma doenca de virus em orquidea. O Biologico **13**: 62. 1947.

REPORT AND ABSTRACTS OF THE NINTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The ninth annual meeting of the Potomac Division of the American Phytopathological Society was held in the Plant Industry Auditorium at Beltsville, Maryland, February 26 and 27, 1952. Approximately 90 members attended the sessions; representing the District of Columbia, Delaware, Maryland, and Virginia. Thirty-five technical papers were presented. The annual banquet was held in the Plant Industry Station cafeteria. Following the banquet Dr. James G. Horsfall spoke on "Chemotherapy in Plant Disease Control."

The following officers were elected to serve during the 1953 meeting: *President*, J. B. DEMAREE; *Vice President*, CURTIS MAY; *Councilor*, C. E. COX.

H. REX THOMAS,
Secretary-Treasurer

ABSTRACTS PRESENTED AT THE MEETING

A technique for obtaining uniform deposition of uredospores on cereal leaves.—BELL, F. H., C. G. SCHMIDT, W. E. MILLER, AND C. H. KINGSOLVER.—Uniform deposition of uredospores on cereal leaves was achieved by exposing leaves in a horizontal position to vertical spore fall. Spores were discharged upwards into a cylindrical metal tower 70 in. high by 30 in. in diameter by means of an instantaneous puff of air. This tower was supported on an exposure chamber 2 ft. high which had a sliding panel at the top. Thirty sec. after spore discharge the panel, upon which the heavier clumped spores settled, was withdrawn and spores allowed to fall on exposed material for 4 min. An average of 5 spores (range, 3.7 to 7.2) of *Puccinia graminis avenae* and 5.3 spores (range, 2.5 to 8.2) of *P. rubigo-vera tritici* per sq. ml. was obtained on coated slides in 2 experiments with each fungus following a discharge of 10 mg. of uredospores. Leaves of cereal seedlings were exposed horizontally by fastening them by means of rubber bands near the base and tip to metal sheets inserted in the soil of the greenhouse pot. In trials with *P. graminis tritici* on wheat seedling leaves, satisfactory uniformity was obtained within experiments, but some variation was encountered between experiments (0.7 to 1.6 spores per sq. ml.)

High-intensity artificial light improves winter expression of stunt symptoms in Mistletoe Chrysanthemums.—Brierley, Philip, M. W. Parker, and H. A. Borthwick.—Mistletoe chrysanthemums systemically infected with stunt express yellow leaf spotting in summer. Such plants develop fewer and smaller spots, or none, during winter under greenhouse daylight supplemented by 6 hr. at night from 100-watt incandescent-filament lamps at an intensity of about 30 to 60 foot-candles at the leaf surface. Expression is improved after 3 wks. if the intensity is increased about threefold by substituting 300-watt lamps. After only two 16-hr. days under a completely artificial source supplying about 2000 foot-candles at the leaf surface symptoms are clearly defined. About 90 per cent of this light comes from a carbon arc and about 10 per cent from incandescent-filament lamps. After 3, 4, 5, 6, and 12 days in this light there is a gradual increase in degree of leaf distortion and in ratio of yellow to green areas. A photoperiod of 12 hr. is very nearly as effective as one of 16 hours. When the same plant is exposed alternately at intervals of a few days to high-intensity artificial light and to winter greenhouse illumination, successive zones of expression and masking develop.

Sugar beet virus yellows in United States.—Coons, G. H. —Virus yellows of sugar beet, a widespread and serious disease of sugar and forage beets in Europe, has now been positively determined as occurring in Michigan, Colorado, Utah, Oregon and California. European specialists on virus yellows have confirmed the similarity of symptoms and have supervised serological tests of suspected plants. Utilizing anti-yellows sera obtained through the courtesy of phytopathological laboratories at Lisse, Netherlands, and Versailles, France, positive diagnostic tests have been obtained with plants collected in the states mentioned. Transmission tests with juice and with aphids have given positive results. The disease known in California as "Salinas yellows," on the basis of both aphid transmissions and serological tests, has been shown to be virus yellows. The virus types so far investigated do not produce veinlet clearing, but cause yellowing and thickening of outer leaves of sugar beet and brittleness characteristic of virus yellows

Reducing the decay hazard of basementless houses on wet sites by means of soil cover.—Diller, J. D.—In a test period of over 9 years, a single layer of asphalt roll roofing on top of the soil under two war emergency houses has been effective in keeping the subfloor timbers dry. During part of the time the vents were closed without any appreciable build-up of moisture content in the timbers, and there was no evidence of condensation moisture. In the meantime various other types of less expensive soil-cover materials have been installed, often in houses where decay was already in evidence and condensation moisture was prevalent, especially during winter. Most of these have proved effective for the 3- to 4-year periods in test, in lowering the wood moisture well below the danger point of 20 per cent, and arrested further development of the wood-decay fungi. Closing vents, especially during cold weather makes the houses warmer. Preliminary results indicate that the usually recommended 2-in. overlap is not necessary, and also that 80 to 90 per cent coverage may give adequate control. Soil cover tests in the northeast, the southeast, and the middle west indicate that soil cover is effective in all regions, and that it is about as effective when no ventilation is provided. The soil cover, however, will not safeguard the temporary buildings against the so-called "water-conducting" fungi, nor against termites.

Six species of Conidiobolus isolated on maizemeal agar canopied with moist plant detritus.—Drechsler, Charles. —Six fungi evidently referable to *Conidiobolus* were isolated from leaf mold and other kinds of plant detritus undergoing slow decay in moist contact with the ground. Their mycelia came to light in a special type of plate culture prepared by affixing detritus with soft agar to a central area on the ceiling of each Petri dish, so that the sterile agar underneath was overhung by a firm canopy of moistened decaying material. One of the 6 fungi appears identical with *C. brefeldianus*. Another gives rise to conidia, mostly about 19μ long and 17μ wide, suggestive of *C. minor*, and in addition produces zygospores about 16μ thick. *C. utriculosus* has not been obtained so far. One species with conidia up to 45μ in diameter is distinguished by a delicate, copiously branched mycelium composed of hyphae 1.8 to 4.5 μ wide, which, unlike the broad sparingly branched hyphae of the several other species, are little given to disjunction into separate segments. All the species so far found producing zygospores are homothallic, conjugation taking place between adjacent segments of the same hypha and between segments of neighboring filaments. The mature zygospore shows a large reserve globule within a narrow parietal layer of protoplasm.

Two species of Basidiobolus widely distributed in leaf mold.—Drechsler, Charles.—Perti-plate cultures of maize-meal agar canopied with leaf mold taken mostly from deciduous woods in Arlington, Virginia, and near Beltsville, Maryland, have yielded many cultures referable to two species of *Basidiobolus*. The two congeners differ little in the vegetative stage, both forming coarse mycelia composed of hyphae about 10 μ wide that soon divide into separate segments commonly 100 to 200 μ long. Asexual reproduction occurs very sparingly in maizemeal agar cultures of one species, but very freely in similar cultures of the other, the devolpement and discharge of conidia in the latter being much as in *B. ranarum* except that rupture of the "basidium" would seem to take place farther upward, since the detached membranous piece is more often of simple conical shape than of the familiar tower-and cupola design. Both species develop numerous zygospores about 30 μ in diameter, always following the production of paired beak-like protuberances. In the first species the thick zygospore wall proper is smooth and intimately fused with the gametangial envelope, whereas in the second the outer contour of the thick zygospore wall is strongly undulate and in part visibly separate from the gametangial envelope. Zygospores of the first species were earlier observed in agar plate cultures planted with discolored or decayed rootlets.

A rapid method for testing susceptibility of pear seedlings to erwinia amylovora.—Dunegan, John C., M. C. Goldsworthy, H. H. Moon, and R. A. Wilson.—Pear seedlings (1,739) from 29 crosses of known parentage at Plant Industry Station were inoculated on April 25, 1951, when they were in bloom, and again on May 7, when the fruit was forming and the young, succulent shoots were 6 to 8 in. long, with an aqueous suspension of *Erwinia amylovora* and an abrasive (pyrophyllite). Three rows of 6-yr.-old Bartlett seedlings (more than 2,000) were left unsprayed as a check on natural blight incidence. Inoculum was applied by a power sprayer developing a pressure of 550 lb. per sq. in. at the pumps, using a single gun with a 12/64th-in. disk opening. All crosses were inoculated in only 60 min. The results from this preliminary experiment are encouraging. Blight symptoms were not observed on the unsprayed seedlings, whereas they developed on 900 of the sprayed seedlings; on 324 the organism invaded the main branches and trunk. In the different groups development of blight appeared to be correlated with the known susceptibility of the parents, crosses having Comice as a parent being particularly susceptible. By practically eliminating the time factor, the method permits valid comparisons of reactions of different crosses, hitherto difficult because of the time required to inoculate so many plants manually.

Post-harvest fungicidal treatments of peaches tested in 1951.—Haller, M. H., W. L. Smith, Jr., M. A. Smith, S. E. Womeldorph, Jr., and W. R. Wright.—Tests with chemicals to control *Monilinia fructicola* and *Rhizopus nigricans* on peaches after harvest were continued in 1951. Each chemical was tested on 2 samples of 50 peaches each, one of which was inoculated with *Monilinia* spores and the other with *Rhizopus* spores. Each test was replicated 4 times. All peaches were wrapped in copper-impregnated paper to inhibit the spread of rhizopus rot to adjacent fruits. Treatments that reduced decay due to *Monilinia* by at least

75 per cent follow: sprays of Orthocide 406 (1 and 2 per cent), Dowicide A (0.5 per cent), Isothan Q 15 (0.5 per cent), and DHA-S (2 per cent) and sulfur dust (90 per-cent). Materials (generally used as 1 per cent sprays) that reduced decay due to *Monilinia* by less than 60 per cent follow: Crag 5379, liquid lime sulfur, wettable sulfur, Seedox, Bisphenol A, nitrophenol carbonate, Clorox, Crag 341C, Crag 531, Crag 658, propylene glycol triethylene, glycol, 8-quinolinol sulfamate and Copper A. Reduction of *Rhizopus* infections was too inconsistent to permit definite conclusions; the following compounds showed some promise (at least 50 per cent reduction): 1 per cent spray with either 8-quinolinol sulfamate, Crag 658, Crag 531 or Copper A. Fumigation with trichloroethane (1 volume of liquid to 7,000 volumes of air) or tetrachloroethane (1-56,000) injured the fruit severely.

An outbreak of Phyllosticta canker and leafspot of soybeans in Maryland.—Jehle, R. A., Anna E. Jenkins, K. W. Kreitlow, and Helen S. Sherwin.—In late August and September, 1951, *Phyllosticta* stem and pod canker, as well as leafspot, was found in epiphytotic proportions in plantings at Salisbury, Wicomico County, Maryland. Up to 50 per cent loss occurred in some fields grown for seed. Stem cankers were gray to almost white, with narrow, purplish to brown borders, 1 to 4 mm. wide by 1 to 12 mm. long. Conspicuous pod and peduncle cankers were also present sometimes causing the valves at the apex of the pod to open, exposing the beans. Many pods were examined in the field and the seed inside found to be small, shriveled and discolored. Occasionally lesions on the pod extended to the seed. Cultures of the fungus were obtained from diseased plants, including seeds, collected at Salisbury and at Beltsville, Maryland. The pathogen was determined as *Phyllosticta sojaecola* Massal. When soybean seedlings were inoculated with the fungus, leaf lesions developed that were identical with those seen in the field.

Influence of light on the morphology and production of conidia in some species of dematiaceae.—Johnson, T. W., Jr., and J. E. Halpin.—Inception of sporulation by *Helminthosporium sativum* and *Curvularia lunata* is 38-49 hr. earlier in colonies exposed to continuous light of 50, 100, 200, 500, and 1000 ft. c. intensity, than in like colonies incubated in darkness. Conidial production increases only slightly as the light is increased. In colonies of *Alternaria solani* incubated in low light intensities, conidial counts are comparable to those from colonies held in darkness. Light of 200 ft. c. or over inhibits conidial production in the same strain. Continuous exposure of *Piricularia oryzae* to these light intensities results in a two-to nine-fold increase in number of conidia when compared with colonies incubated in the dark. An isolate of *Helminthosporium teres* did not sporulate either in light or dark. All species gave comparable results when propagated on 6 different media, with maximum conidial production falling at the 200 or 500 ft. c. level. The magnitude of conidial increase is markedly affected by substratum, but the influence of depth of medium, humidity, pH, or quality of light, is negligible. On some media, absence of light or low intensity effects shortening and abortion of conidia of *H. sativum*. Regardless of substrate, the conidia of *A. solani* and *P. oryzae* become successively more slender and elongate as light intensity is increased. Presence or absence of light has little effect on the morphology of the conidia of *C. lunata*.

Studies on inoculation of carnations with pseudomonas caryophylli.—Lewis, C. E., R. N. Stewart, and W. F. Jeffers.—To facilitate breeding and selection of wilt-resistant varieties of carnations, a study was undertaken to develop effective inoculation techniques. Conditions favorable for high infection percentages, together with a short incubation period, were determined, so that large numbers of cuttings and seedlings could be tested quickly for susceptibility to bacterial wilt. Seedlings or cuttings were inoculated by dipping the base in a bacterial suspension, by dropping it on topping wounds, by hypodermically injecting it into stems, and by pouring it on the soil. Rooted cuttings, dipped in inoculum and planted in soil at 95° F., gave 100 per cent infection in an average of 13 days. This method of inoculation was also the most effective for seedlings. Air humidity and temperature had no important effect in contrast with soil temperature, which was correlated positively with infection percentage and negatively with incubation period. Wounds were necessary for entrance of the pathogen. Those received at transplanting were sufficient, but enough healing occurred in 7 days to prevent infection. Minimizing root injury by more careful handling of plants might decrease infection in the bed. Use of plant bands for rooted cuttings offers a possible method of reducing injuries at transplanting.

A new physiologic race of rust (Uromyces Phaseoli-Typica Arthur) causing losses to beans in Maryland.—Marcus, Carlton P., Jr.—In 1948, 1949 and 1950 severe infections of rust (*Uromyces phaseoli-typica*) caused considerable losses to late plantings of beans in Kent County, Maryland. Investigations using the differential series, as described by Harter and Zaumeyer, have proven that a mixture of 2 physiologic races of the organism were responsible for the epiphytotic. By making single pustule isolations the components were separated. They proved to be race number 26, and a new race designated as number 82. It is thought that the fungus overwinters in the uredinial stage under Maryland conditions. Indications are that a small number of uredospores survive the winter and build up a sufficient number to cause an epiphytotic by the late summer. No aecia have been found under field conditions in Maryland. A 1951 experimental dusting program was not successful because the rust did not appear, but the unseasonably dry weather may have been responsible. However, it is possible that the rust can be controlled by dusting with sulfur at weekly intervals beginning in July before any signs of rust appear. The sulfur is applied at the rate of 30-35 lbs. per acre.

Relation of wound periderm in gladiolus corms to penetration by Fusarium oxysporum f. gladioli.—Marshall, B. H., Jr.—The effect of optimum conditions for wound periderm formation on the susceptibility of cut surfaces of gladiolus corms to penetration by *Fusarium oxysporum* f. *gladioli* was tested. Picardy corms were surface-sterilized, sliced into quarters, placed in moist chambers, and incubated at 85° F. One group of corms was inoculated immediately with a suspension of Fusarium spores applied directly to the cut surface and one group was inoculated each day thereafter for 7 consecutive days. Samples of all corms were stained and examined daily for 1 wk. after inoculation. The effectiveness of wound periderm in inhibiting penetration by Fusarium was clearly evident. Considerable rotting occurred in the corms inoculated immediately after cutting, and there was progressively less rot in those inoculated after 1 to 4 days. Fusarium failed to penetrate corms inoculated after 5, 6, and 7 days' exposure to conditions favorable for wound periderm formation.

Screening organisms for antagonism to Ceratostomella fimbriata. Martin, J. P., Jr., and W. F. Jeffers. Of various organisms tested for antagonism to *Ceratostomella fimbriata*, *Bacillus brevis* No. 9999 A.T.C., *Bacillus polymyxa* No. 10401 A.T.C., *Streptomyces griseus* No. 10137 A.T.C., *Penicillium claviforme* No. 9257 A.T.C., *Penicillium janczewskii* No. 9403 A.T.C., and 9 unidentified *Streptomyces* isolates showed antagonism. Of these unidentified isolates, SL 17, SL 20, and SL 25, were unusually active. These isolates, apparently, are physiologically different. A single *in vitro* method has been used to test for antagonistic action. The potential antagonist was placed in the center of a petri plate containing Wellman's modification of Tochinai's solution agar and the test organism was placed on either side. Flattening of the characteristic circular growth pattern of *C. fimbriata* indicated antagonism. Soil isolate, SL 25, in amounts of malt extract and yeast dextrose agar ranging from 10cc to 50cc per petri plate showed no change in antagonistic action. No visible change in antagonism was noted when the culture medium was adjusted to different

pH values. A crude autoclaved extract from SL 25 greatly retarded growth of *C. fimbriata* even when diluted with four parts of water. There was no correlation between pigment production and antagonistic effect in the isolates of *Streptomyces*. Isolate SL 25 was also found to be antagonistic to *Fusarium oxysporum f. batatas* and *Ceratostomella ulmi*.

Transmission of a tobacoc leaf curl to other hosts.—Morgan, Omar D.—A number of methods were used in an attempt to transmit a leaf curl disease of tobacco found in Maryland in 1950. Thirty-six trials were made to transmit it by rubbing sap from diseased plants on leaves of other Maryland tobacco strains; results were negative. Dodder transmission was tried by attaching dodder to diseased tobacco—then to healthy tobacco, tomato and jimson weed; results were negative. Seed from diseased plants were germinated and 200 seedlings were observed for 6 wk; no leaf curl appeared. Ten of the seedlings were transferred to pots and observed 5 mo.; leaf curl did not appear. The disease was graft-transmitted to 2 Turkish Samsun variety tobacco plants, to 8 T. I. 448-A variety tobacco, and to 3 Catterton strains of Maryland tobacco. The disease was graft-transmitted to 3 tomato plants, variety Marglobe, and once to *Datura stramonium*. The disease was transmitted from Turkish to Turkish, from 448-A to 448-A, and from tomato to tomato by grafting. In each case of transmission, there was a typical curling downward of leaf, crinkling of intervenal tissue and knotting and twisting of veins, shortening of internodes and dwarfing. The cause of the disease is probably a virus which resembles the leaf curl of tobacco.

A preliminary screening of the genus Nicotiana for resistance to tobacco anthracnose.—McGrew, John R.—Forty-two of the 58 species of *Nicotiana* recognized by Goodspeed, and 14 commercial varieties of tobacco were tested for susceptibility to tobacco anthracnose. The *Colletotrichum* sp. used was isolated from typical stem lesions on a diseased plant from Calvert County, Maryland. An aqueous suspension of conidia harvested from colonies of the pathogen grown on Czapek's agar medium was sprayed onto young seedlings, which were then held for from 72 to 96 hr. at 22° C. at 100 per cent relative humidity, and then transferred to the greenhouse. Three plants of each species were inoculated and a fourth noninoculated plant used as a control. When fewer plants were available, an excised leaf technique, which gave comparable results, was used. Rating of susceptibility was on the basis of percentage of leaf area affected, and severity of the disease, under conditions of experimental inoculation was greater than generally found under natural conditions on comparable varieties. All commercial varieties tested were found to be equally susceptible. Species which showed somewhat greater resistance than these were: *N. Goodspeedii, N. nesophila, N. exigua, N. repanda, N. Knightiana, N. glauca,* and *N. occidentalis.* Seven species found to be highly resistant were: *N. nudicaulis, N. longiflora, N. Langsdorffii, N. alata, N. trigonophylla, N. Debneyi,* and *N. sylvestris.*

Influence of potassium nutrition of the host on the reaction of lima bean plants to infection by a root-knot nematode, Meloidogyne incognita.—Oteifa, Bakir A.—Henderson lima bean plants were grown in sand culture inoculated with the root-knot nematode, *M. incognita.* Three potassium treatments: low, optimum and excessive concentrations, were employed. Three levels of nematode inoculum were used: 0,50 and 200 egg masses. Results showed that root knot caused a general reduction in plant growth, as determined by total fresh and total dry weights. This reduction is correlated with the increase of inoculum. Chemical analysis showed infected plants had lower amounts of nitrogen, phosphorus, calcium, magnesium and were very low in potassium as compared to controls. Infected plants supplied with excessive amounts of potassium made almost normal growth and maintained mineral levels comparable to the controls. Rate of reproduction of this nematode was found to increase correspondingly with the potassium supply. Thus it appears that nematode damage is correlated with the potassium available. The root-knot nematode with its high potassium requirements appears to interfere with the potassium nutrition of its host, causing nutritional injury.

Present status of the peach anthracnose problem in Georgia.—Petersen, D. H., and John C. Dunegan.—The circumstantial evidence implicating blue lupine as the source of *Glomerella cingulata* spores causing peach anthracnose appear to have been further augmented in 1951. This peach disease, after increasing in severity each year from 1947 to 1950, virtually disappeared in the central-Georgia peach section in 1951 following the destruction of commercial lupine plantings by low temperatures. However, attempts to produce experimentally a severe outbreak of anthracnose in peach trees by spraying them with *G. Cingulata* spores failed to give consistent results. This suggests the possibility that some factor other than the absence of lupine might have been responsible for the lack of anthracnose on peaches in 1951. The alleged correlation between the anthracnose on blue lupine and that on peach is still based solely on circumstantial evidence and remains to be demonstrated experimentally. Unfortunately, the opportunity for an experimental test may not exist in 1952, for the lupine has been seriously injured again by low temperatures, according to reports from Georgia.

Studies on the control of root-knot nematodes (Meloidogyne Spp.) with Systox spray (E-1059), an organic phosphate insecticide.—Sasser, J. N.—Systox concentrations of .005, .01, .02, .03, .05, .1, .5 and 1.0 per cent when applied as a soil drench at the rate of 300 ml. per 5-inch pot with tomato seedlings greatly reduced infection and reproduction of root-knot nematodes (*Meloidogyne* sp.). Concentrations of .5 and 1.0 per cent proved phytotoxic. Cucumber seedlings grown in 5-inch pots of infested soil drenched with 300 ml. of Systox at concentrations ranging from .05 to .1 per cent 1 wk. prior to seeding, made significantly better growth than the controls. Concentrations of .2 to .5 per cent caused stunting. Root knot was controlled at the .1 per cent and higher concentrations. Tomato seedlings which had absorbed Systox from treated sterile soil, when transplanted to infested soil became severely infected. Larval hatching from egg masses placed in Systox concentrations of .05, .1 and .2 per cent was inhibited for 1 wk. but hatched readily when removed from Systox solutions and placed in tap water. Egg masses treated as above when placed in pots with tomato seedlings caused severe infection in 39 days. Systox solutions of .1 and .2% in contact with hatched larvae inhibited their movement, but failed to kill them for several days. Systox in the soil apparently acts as an inhibitor of hatching of root-knot nematodes and is lethal upon long exposure.

Powdery scab, a precursor for the late blight infection of blight-immune potato tubers.—Schultz, E. S.—In Maine during the 1951 epiphytotic of late blight, caused by *Phytophthora infestans,* blight-immune Kennebec potato tubers contracted late blight in soil infested with the powdery scab organism, *Spongospora subterranea.* Observations of blighted tubers disclosed that infected areas were closely associated with the powdery scab lesions, which apparently served as invasion loci for the late blight fungus. Examination of tubers affected with powdery scab at harvest in a blighted field revealed that about 30 per cent of the tubers were infected with late blight, whereas none of the clean or scab-free tubers was infected. Experience has shown that the late blight fungus invades blight-immune tubers through injuries or wounds on the tubers. The powdery scab sori that are not suberized apparently play the same role as injuries in serving as invasion sites for the late blight fungus in blight-immune potato tubers.

Electron microscope studies of purified tobacco ring spot virus.—Senseney, C. A., P. R. Desjardins, and G. E. Hess. —This work was done with the electron microscope to verify shape and particle size of tobacco ring spot virus reported in the literature. The virus was purified by differential centrifugation following extraction from frozen tobacco plant leaf tissue. The specimens were prepared for

electron microscopy using the protein monolayer technique and the spray drop method. The virus was found to be approximately spherical with a diameter of 18.3 mμ by shadow measurements and 21.8 mμ by measurements perpendicular to the direction of shadow. These values are in agreement with those found by ultracentrifugation and ultrafiltration studies of purified preparations and ultrafiltration studies on nonpurified virus as described in the literature. studies on nonpuried virus as described in the literature. Both values here reported are less than the 26 mμ reported for electron microscope studies of an unpurified preparation.

Decay of nonheated and heat-injured potatoes as influenced by storage temperature.—SMITH, WILSON L., JR.—Potatoes artificially injured by exposure to heat until the temperature under the skin surface approached 120° F. were placed at 40°, 50°, 60°, 70° and 90° for an initial week and then at 90° for an additional week. Nonheated tubers also were stored at the same temperature sequences. Results at the end of the first week showed that low-temperature storage did not prevent development of symptoms of severe heat injury. During this period decay occurred only in the 70° and 90° storages, the greater percentage at the higher temperature. At the end of 2 wk. the decay percentages for the heated potatoes were 53, 40, 35, 27 and 79 and for the nonheated were 25, 20, 12, 1, and 16 when the initial storage temperatures were 40°, 50° 60°, 70°, and 90°, respectively. The experiments therefore indicate that conditioning tubers at 60° or 70° does materially reduce decay at high temperature, but that less reduction occurs at the same high temperature if the tubers are conditioned at 40° or 50°.

The development of cercospora leaf spot resistant monogerm sugar beets.—STEWART, DEWEY.—F_1 seedlings, leaf spot resistant multigerm × susceptible monogerm SLC 101, were received from Dr. V. F. Savitsky who had introduced the monogerm race of sugar beets in 1948. Roots of leaf spot resistant varieties were supplied to him from selections made under severe disease exposure on the Plant Industry Station. Since the monogerm character in SLC 101 is recessive and conditioned by a single pair of alleles, the F_1 plants produced multigerm fruits. The F_2 monogerm segregants showed striking variation in vigor and seed size. The F_3 monogerm progenies and the parental varieties were grown under leaf spot exposure of medium severity. The disease reactions of the parental varieties were recovered in certain of the F_3 lines, while other F_3 lines showed marked segregation for disease resistance. Eight of the most resistant and most vigorous F_3 lines from F_2 plants with large fruits have been selected for increase and hybridizations.

A correlation between russet and storage shrinkage of Starking apples in West Virginia.—TAYLOR, CARLTON F.—Russeting of apple fruits frequently follows the application of many pesticidal sprays. The depreciated market appearance of affected fruits is well recognized; the following data show an increased shrinkage of russeted apples during storage. In 1950 Starking apples from an apple scab-fruit finish spray experiment were stored 6½ mo. at 31° F. They then were sorted into 3 degrees of russet: none, slight, and moderate to severe (the latter class were those apples not acceptable in the 1950 U.S. 1 grade). These lots were cross-sorted into 3 shrinkage classes: none, slight (fine shrinkage lines detectable), and moderate to severe (loss turgidity in varying degrees). Apples, 100 each from 7 different spray treatments, were available. In composite sample, 437 of the 700 were free from visible russet. Of these, 433 (99 per cent) were free from shrivelling, 3 (1 per cent) were slightly shrivelled, and 1 was moderately shrivelled. The 153 apples with slight russet showed no shrivelling on 112 (73 per cent), slight on 28 (18 per cent), and moderate to severe on 13 (9 per cent). The 110 with moderate to severe russet had 18 (16 per cent) not shrivelled, 36 (33 per cent) slightly shrivelled, and 56 (51 per cent) with moderate to severe shrinkage.

Another strain of alfalfa mosaic virus systemically infectious on beans.—ZAUMEYER, W. J.—A new strain of alfalfa mosaic virus producing primary necrotic, systemic necrotic, and severe-mottle symptoms on beans was isolated from alfalfa in Idaho. These symptoms differ from those produced by previously reported strains of alfalfa mosaic virus except the yellow dot virus. The latter produces more veinal necrosis than primary spots and the systemic-mottle is milder than that of the new strain. Cucumber and red clover are susceptible to the yellow dot virus but not to the new virus. The host range of the new virus is similar to that of other strains of alfalfa mosaic virus; however, cucumber, pepper, petunia and red clover not infected by it are susceptible to many of the other strains. On Pinto bean, the primary necrotic lesions produced by the new strain are irregular in outline, 3 to 4 mm. in diameter, reddish brown, with light green centers. Veins, petioles, and stems become necrotic, trifoliate leaves are mottled and show necrotic lesions. Later, large yellow blotches occur. On other varieties like Bountiful, yellow chlorotic spots appear on the inoculated leaves a few days after inoculation. Later these and the trifoliate leaves become mottled but no necrosis occurs. The physical properties conform with the type strain. It was inactivated at 65° to 70° C. in 10 min. and in 72 hr. at 18° and withstood a dilution of 4000 ×.